SELENIUM TOLERANCE IN SHEEP AND SELENIUM SUPPLEMENTATION METHODS FOR BEEF CATTLE

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2004

To our Heavenly Father and my loving family.

ACKNOWLEDGMENTS

The author wishes to express the utmost gratitude to Dr. Lee R. McDowell, chairman of the supervisory committee, for his guidance, patience, encouragement and direction throughout the Ph.D. program and during preparation of this dissertation. Appreciation is given to Drs. Tim Marshall, Claus Buergelt, and Richard Weldon for service and dedication on the graduate committee and for advice and understanding. Special thanks are extended to Dr. McDowell for his unselfish attitude and including me on several experiments, publications, and educational opportunities. Gratefulness beyond words is in order to Dr. McDowell for showing me that one will get further by lifting others up than by putting anyone down. Dr. Todd Thrift deserves thanks for his willingness to listen and provide both professional and personal advice.

A huge debt of gratitude and appreciation is extended to Mr. Bert Faircloth, Mr. Charles Stephens, Mr. Steve Chandler, Mr. Jesse Savell, and Mr. Brantley Ivey at the Santa Fe Beef Unit for their help in conducting the experiments and collecting data. Mr. Dean Glicco deserves particular thanks for his care of the experimental animals and for becoming a trusted friend and confidant to the author. The author thanks Ms. Nancy Wilkinson for sharing her invaluable knowledge of lab procedures and for her patience in instruction of laboratory techniques. Mrs. Lorraine McDowell deserves thanks for her work with electron microscopy on animal tissues.

Fellow graduate students Deke Alkire, Carlos Alosilla, Bradley Austin, Nathan Krueger, Edgar Rodriguez, and Oswaldo Rosendo deserve thanks for their help with sample collection. Also, Eric Matsuda-Fugisaki provided some needed assistance in the laboratory and is much appreciated. Likewise, appreciation is extended to all graduate students in the Animal Sciences Department for support and camaraderie during this program of study. Pam Gross deserves recognition for her unyielding willingness to help others and her wonderful attitude.

United States Sugar Corporation and its employees deserve thanks and recognition for their donations of liquid feeds. A note of appreciation goes to Dr. Jon Nelson and Southeastern Minerals, Bainbridge, Georgia, for donation of mineral supplements, to Flint River Mills for transportation of mineral supplements, to Alltech, Nicholasville, Kentucky, for donation of Sel-Plex®, and to Mr. Dane Bernis for preparation of experimental diets.

The author wishes to acknowledge, though not necessarily in a positive manner, Hurricanes Charley, Frances, Ivan, and Jeanne. In the worst hurricane season in many decades, the storms caused chaos across the state of Florida by damaging property and generally disrupting daily life.

Last, but certainly not least, the author wishes to thank Ms. Rachel Van Alstyne for her assistance with laboratory analyses and for her unselfishness and dedication as a friend and colleague. She has truly been the kind of friend that a friend would like to have.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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December 2004

Chair: Lee Russell McDowell
Major Department: Animal Sciences

A series of experiments to evaluate and compare methods, sources, and dietary levels of selenium was carried out utilizing sheep and cattle. Experiments using sheep were conducted to gather further data on 1) the tolerance of dietary inorganic Se by ewes during lamb production, 2) the effects of high levels of dietary Se fed to ewes on their lambs, and 3) the tolerance of organic or inorganic Se by mature wethers. A cow-calf herd was used to evaluate and compare effects of using different forms of dietary or parenteral Se on weight change and blood, milk, and liver Se concentrations of beef cows and their calves. In ewes fed Se, as sodium selenite, above requirements, Se concentrations in blood, wool, and soft tissues generally increased ($P < 0.05$) as dietary Se increased. Ewes tolerated up to 20 mg/kg dietary Se without suffering from toxicosis. Lambs born to ewes receiving high levels of dietary Se had increased plasma Se ($P < 0.05$) as Se in ewe diets increased. No signs of Se toxicosis were observed in lambs regardless of Se concentration in the ewe diets. Wethers, fed up to 40 mg/kg Se as

sodium selenite or Se yeast for 60 wk, had Se concentrations in serum, whole blood, wool, and soft tissues which increased as dietary Se increased ($P < 0.05$). In general, Se yeast vs selenite was more effective at increasing Se in blood, wool, and soft tissues ($P < 0.05$). Enzyme activity and histopathological evaluation of soft tissues from ewes and wethers indicated no evidence of Se toxicosis. From the two sheep experiments, maximum tolerance for both forms of dietary Se is greater than 40 mg/kg. Cows receiving Se supplementation as Se yeast maintained adequate concentrations of Se in plasma, whole blood, and liver and generally had higher ($P < 0.05$) concentrations than cows receiving inorganic Se. Calves from cows receiving Se via free-choice minerals had higher ($P < 0.05$) weight gains than from cows receiving injectable selenate. Calves whose dams received Se yeast generally had higher Se ($P < 0.05$) in blood and liver.

CHAPTER 1
INTRODUCTION

Selenium (Se) has had a long and storied history in animal nutrition. Since its discovery, at the bottom of a vat of sulfuric acid, by Jöns Jacob Berzelius, a Swedish chemist, in 1817, Se has played the role of toxic element, essential nutrient, carcinogen, and contributor in cancer prevention. However, it seems that selenium's greatest legacy is one of a toxic agent to livestock. As early as 1295, Se was documented as detrimental as Marco Polo described a poisonous plant which, when eaten by horses, caused their hooves to drop off (Komroff, 1926). Likewise, a U.S. Army surgeon, stationed at Fort Randall in 1856, described much the same conditions afflicting horses in the Nebraska Territory (Madison, 1860). Selenium was identified as the principal toxic agent in conditions described as "blind staggers" and "alkali disease" throughout Wyoming and the Dakotas. In 1957, Se was shown to prevent liver necrosis in rats and afterward was deemed an essential nutrient (Schwarz and Foltz, 1957). Though much of the world is afflicted with Se deficiency, supplementation of Se using dietary or parenteral forms will generally resolve the problem. Selenium toxicities require more effort but can be successfully combated, by not overdosing livestock with supplemental Se, monitoring Se content of feedstuffs, and by using certain animal management techniques.

With its many implications as a toxic element, the use of Se, as a supplement to livestock, garners much caution from feed manufacturers, animal scientists, and nutritionists. The current estimate of the maximum tolerable level for dietary Se in

domestic animals is 2 mg/kg (National Research Council [NRC], 1980). This estimate does not consider differences inmetabolism of Se by different species and makes no differentiation in the maximum tolerable level for the different chemical forms of Se, such as Se yeast or sodium selenite. Previous research has shown that the absorption of an oral dose of inorganic Se differs between ruminant and monogastric species (Wright and Bell, 1966). Likewise, studies in cattle and swine have shown a marked difference in the efficacy of organic vs inorganic Se to increase blood, milk, and tissue Se concentrations (Pehrson et al., 1999; Kim and Mahan, 2001; Gunter et al., 2003). Furthermore, some evidence exists to suggest that the maximum tolerable level of Se for livestock is grossly underestimated and to discredit the notion that the range between optimal and toxic levels of Se is narrow (Glenn et al., 1964a; Kim and Mahan, 2001; Cristaldi et al., in press).

To further the body of knowledge in this subject area, a series of experiments were carried out with sheep and cattle. Experiments using sheep were conducted to gather further data on 1) the amount of dietary inorganic Se that can be tolerated by ewes during lamb production, 2) the effects of Se supplementation to ewes on their lambs, and 3) the amount of organic or inorganic Se that can be tolerated by mature wethers. A cow-calf herd was utilized to evaluate and compare effects of using different forms of dietary or injectable Se on body weight change and blood, milk, and liver Se concentrations of beef cows and their calves.

CHAPTER 2
REVIEW OF LITERATURE

Benefits of Selenium Supplementation to Livestock

Selenium's role in animal nutrition was drastically changed when it was identified as the third factor involved in preventing liver necrosis in rats (Schwarz and Foltz, 1957). After this first evidence for the essentiality of Se, benefits for many other species were discovered. Patterson et al. (1957) demonstrated that Se would prevent exudative diathesis in chicks and Eggert et al. (1957) showed that hepatosis dietetica (liver necrosis) could be prevented in swine by feeding Se. In calves and lambs, Se was successful in preventing white muscle disease (WMD), a condition also known as nutritional myodegeneration (Hogue, 1958; Muth et al., 1958).

Corah and Ives (1991) reported that insufficient Se could be linked to a variety of disorders in beef cattle. Among the reproductive disorders observed were retained placenta, infertility, abortions, births of premature, weak, or dead calves, cystic ovaries, metritis, delayed conception, erratic estrus periods, and poor fertilization. In addition to problems in reproduction, a condition known as "ill-thrift" has also been reported in cattle (Corah and Ives, 1991; Underwood and Suttle, 1999) and also affects sheep. "Ill-thrift" is defined as a syndrome that includes subclinical growth deficit, clinical unthriftiness with rapid loss in weight, as well as some mortality. Selenium deficiency has also been linked to cases of mastitis in dairy cattle that occurred more frequently and lasted longer than mastitis in cattle with adequate Se intake (Smith et al., 1985).

Perhaps Se is best known for its role as an essential constituent of glutathione peroxidase (Rotruck et al., 1973) and four Se-dependent glutathione peroxidases have been identified and designated as glutathione peroxidase 1, 2, 3, and 4 (Lei et al., 1998). These four enzymes benefit animal health by protecting cellular and subcellular membranes against oxidative damage. Also, it appears that Se-dependent glutathione peroxidases provide a second line of defense against peroxidation of vital phospholipids (McDowell, 2003). Vitamin E provides the first line of defense against the peroxidation of phospholipids in membranes.

Adequate dietary or supplemental Se is an effective way to combat the aforementioned problems in growth and reproduction of livestock. Likewise, in the presence of adequate Se, the glutathione peroxidase system works in synergy to protect animal cells against lipid peroxidation. In general, livestock species have minimum requirements of dietary Se which range from 0.05 to 0.30 mg/kg (McDowell, 2003). There are numerous examples throughout the scientific literature that cite benefits in growth, reproduction, and prevention of WMD and other anomalies due to adequate dietary or supplementary Se.

The dietary Se requirement for all classes of sheep ranges from 0.10 to 0.20 mg/kg (NRC, 1985). However, the minimum dietary Se level necessary to prevent WMD varies as reported in the literature. Oldfield et al. (1963) reported that 0.06 mg/kg was the minimum dietary Se level required to prevent WMD in lambs. However, researchers in New Zealand indicated that lambs had normal growth and remained free of clinical signs of Se deficiency when grazing pastures containing 0.03 to 0.04 mg/kg (Hartley and Grant, 1961). Oldfield et al. (1963) further reported that ewes fed a ration containing

only 0.02 mg/kg Se gave birth to lambs with WMD, but by supplementing 0.10 mg/kg Se in the ewe diet, WMD was prevented consistently. It is suggested that at least some of the variation in the Se requirements necessary to prevent WMD is due to sparing or interfering nutrients, such as Vitamin E or sulfur, and that differences also reflect Se losses due to drying, as well as analytic error (McDowell, 2003). Maas et al. (1984) suggested that even in cases of Se deficiency, lambs returned to a normal Se status with one or two i.m. injections containing 1 mg Se and 68 IU of vitamin E. Selenium supplementation to ewes at a level of 2.25 mg/d reduced both the incidence and severity of WMD in white-faced lambs (Gardner and Hogue, 1967).

Selenium supplementation has been reported to have some effects on growth and rate of gain in sheep and cattle. Spears et al. (1986) reported increased summer gains in calves that received Se and vitamin E supplementation vs those calves receiving no Se supplementation. Likewise, Perry et al. (1976) reported a 10% increase in ADG of steers when feedlot diets were supplemented with 0.1 mg/kg Se. Furthermore, an 8% increase in ADG of finishing beef cattle was again reported when 0.1 mg/kg Se was added to the diet (Burroughs et al., 1963). Increases in BW gains of 20% were attained when Friesian heifer calves were supplemented with Se at a rate of 3 mg/d (Wichtel et al., 1996). In lambs, data from Oldfield et al. (1963) indicated that lambs with the lowest blood Se had the lowest BW at six wk of age. However, reports of a positive response in growth or BW gain are inconsistent in sheep and cattle. Ammerman et al. (1980) reported no differences in weaning weights of calves nursing Se supplemented mothers vs calves whose dams had received no supplemental Se. Hereford × Angus calves showed no difference in ADG from birth to weaning due to supplemental Se (Castellan et al., 1999).

Likewise, ADG, feed consumption, and gain:feed were not affected by supplementation of 0.1 mg/kg dietary Se fed for 13 wk or 0.2 mg/kg dietary Se fed for six wk in separate studies, two using sheep and one using cattle (Ullrey et al., 1977).

Newborn and suckling calves and lambs can receive Se via their dams from either maternal transfer or increased Se in milk. Recent studies indicate that blood Se in newborn calves can be increased through Se supplementation of their dams (Abdelrahman and Kincaid, 1995; Gunter et al., 2003; Valle et al., 2003). Likewise, positive correlations between Se concentration in dam's milk and Se concentration of calf whole blood have been observed in calves up to 70 d of age (Pehrson et al., 1999). Evidence also exists that milk Se can be increased by level and duration of Se supplementation in lactating cows (Conrad and Moxon, 1979). Like blood and tissue, milk Se is affected by dietary Se level (Conrad and Moxon, 1979; Givens et al., 2004) and Se readily crosses the placenta to the fetus (Van Saun et al., 1989). A strong relationship of dietary Se to Se in milk of dairy cows was reported with up to 18.08% of dietary Se being recovered in milk (Maus et al., 1980). Koller et al. (1984) supplemented first-calf Hereford heifers with dietary Se and concluded that Se readily crosses the placenta in beef cattle. Furthermore, those authors added that low Se concentrations in the blood of dams could cause the fetus to gather more Se and result in fetal blood Se that is higher than that of the mother. In sheep, Cuesta et al. (1995) showed increased colostrum Se from ewes receiving supplemental Se and that milk Se was higher after one mo of lactation. Also, Jacobsson et al. (1965) concluded that Se administered to ewes could be transmitted to lambs through the placenta and the milk after a study using radio-labeled sodium selenite and selenomethionine. In a study utilizing swine, Wuryastuti et

al. (1993) documented the importance of Se and vitamin E for maintaining immune function in livestock. Those authors measured immune responses of blood, colostrum and milk leukocytes of sows and concluded that greater phagocytic and microbicidal activity could be realized in milk and colostrum through supplementation with Se and vitamin E.

Reproductive problems in beef cattle such as retained placenta, infertility, abortions, births of premature, weak, or dead calves, cystic ovaries, metritis, delayed conception, erratic estrus periods, and poor fertilization may be successfully overcome with Se supplementation (Corah and Ives, 1991). Awadeh et al. (1998a) concluded that Se intakes of pregnant cows could be an important factor in weak calf disorders and that passive immunity and heat production by newborn calves using brown adipose tissue could both be influenced by maternal Se intakes. A Se deficiency in the diet of dairy cattle was reported to be a contributor to a high incidence of retained placentas (Trinder et al., 1973). Data from studies using dairy cows have shown that supplemental Se and vitamin E to animals receiving Se deficient diets are beneficial in decreasing the incidence of retained placentas (Julien et al., 1976; Hemken et al., 1978). Smith et al. (1988) studied the effects of Se on disease resistance in dairy cattle and concluded that many dairy herds have inadequate dietary intakes of Se and vitamin E. Those authors added that insufficient intakes of these nutrients could result in increased cases of mastitis, metritis, and retained placenta, and recommended Se supplementation at a level to maintain blood Se at a minimum of 200 μg/L. Weiss et al. (1990) studied the relationships between Se and mammary gland health in commercial dairy herds and

concluded that high serum Se concentrations were associated with reduced rates of clinical mastitis and low somatic cell counts in the milk tank.

Reproductive problems such as increased services per conception or increased calving interval which could, at least in part, be attributed to male fertility may also be improved by Se supplementation. Heimann et al. (1981) showed that the pituitary gland and reproductive tissues exhibited higher Se concentrations than many other body tissues. Julien and Murray (1977) reported that percent motility in bovine spermatozoa increased significantly as concentration of Se in sperm increased. Thus, supplemental Se may have a positive effect on sperm quality and ultimately on male fertility. However, Segerson and Johnson (1981) observed no differences in sperm number, viability, or Se content from Se supplemented bulls compared to sperm from unsupplemented controls.

Methods of Selenium Supplementation to Livestock

The benefits of Se supplementation to livestock are many and Se deficiencies are easily combated with adequate Se supplementation. Several methods of Se supplementation exist and successful uses of all methods have been reported. The method of Se supplementation chosen by livestock producers may be dependent on factors such as Se content of soils, local grains and forages, species produced, class of livestock and stage of production, facilities for animal handling, as well as knowledge, previous experience, and personal preference.

Many areas of the United States have Se deficient soils (McDowell, 2003) and thus produce grains and forages which are low in Se. Likewise, many regions of the world have been mapped as Se deficient and may benefit from the administration of Se to livestock (Oldfield, 2002). In a survey of blood Se status in beef cattle encompassing more than 250 herds in 18 states in several regions of the U.S., more than 18% of cattle

were classified as marginally deficient (51 to 80 μg/L) or severely deficient (≤ 50 μg/L) in blood Se (Dargatz and Ross, 1996). Percentages of cattle classified as deficient varied with region of the country. Herds in the Central U.S. had the least occurrence of Se deficiency, while the Southeast, including Florida, had the greatest incidence of Se deficiency at more than 40%. Stowe and Herdt (1992) also suggest that many cattle in the U.S. are in a state of Se deficiency.

Selenium supplementation to livestock is accomplished using three or four primary methods. Addition of Se to livestock feeds and/or minerals, use of injectable Se preparations (usually in combination with vitamin E), use of sustained release intrareticular Se supplements, and possibly the use of seleniferous grains or forages grown on high Se soils (Ammerman and Miller, 1975) are the methods most often used to supplement Se. One additional option to increase Se intake of livestock is the use of Se containing fertilizers on forage and pasture (Valle, 2001). The addition of Se to feedstuffs was not an option until 1974 when the Food and Drug Administration (FDA) allowed for supplementation of up to 0.1 mg/kg Se as selenite or selenate for swine and poultry (Schmidt, 1974). An amendment to this FDA order allowed the use of supplemental Se for sheep in 1978 and a subsequent amendment in 1979 allowed for use in dairy and beef cattle. Currently, use of 0.3 mg/kg dietary Se is approved for supplementation in poultry, swine, sheep, and cattle (McDowell, 2003).

Regardless of method chosen for Se supplementation, Se deficiencies are more easily combated than are toxicities, which generally require more animal and/or pasture management. In sheep and beef cattle production systems, producers most often choose to use injectable Se products or supplement Se through free-choice mineral mixtures.

Judson et al. (1991) evaluated long-acting Se treatments for ewes and lambs in a 200 wk experiment. Those authors reported that a 100 mg injection of barium selenate was more effective at increasing blood Se of ewes and their lambs than was an intraruminal Se pellet or no Se supplementation. Data show a near five-fold increase in blood Se from lambs from injectable selenate treated ewes vs lambs from unsupplemented dams. Norton and McCarthy (1986) evaluated injectable Se products for prevention of WMD in lambs and reported increased plasma and milk Se in ewes that received the injectable Se vs unsupplemented controls. Likewise, those authors showed increases in lamb plasma Se due to the frequency of use of injectable Se. In a series of University of Florida studies, the use of injectable Se, as sodium selenite and barium selenate, in a cow-calf herd was evaluated and compared to inclusion of organic Se in free-choice mineral mixtures (Valle et al., 2002; 2003). Those authors reported that, in general, injectable Se as selenate and selenite affected plasma, liver, colostrum, and milk in a similar manner. Though the injectable products did increase Se levels in blood, milk, and tissue compared with blood, milk, and tissue Se concentrations from unsupplemented animals, both injectable forms of Se were generally less effective than the addition of organic Se to free-choice minerals. The calves born to and suckling cows that received injectable Se had plasma Se concentrations which were similar to plasma Se concentrations of calves from unsupplemented dams. Selenium supplementation via free-choice minerals proved more effective at raising and maintaining Se status of Florida beef cows and their calves.

Gunter et al. (2003) compared effects of Se supplementation as sodium selenite or Se yeast added to free-choice minerals on performance and Se status of beef cows and calves in Arkansas. Mineral mixtures were formulated to contain 26 mg/kg Se and were

offered free-choice. No differences in performance between unsupplemented controls or cattle receiving either form of Se were observed. However, differences in blood Se of supplemented vs unsupplemented cattle were reported. Likewise, Se yeast treated cows and their calves had higher blood Se than cows and calves receiving selenite Se. Those authors concluded that calves are at risk for Se deficiency if their dams are not supplemented with Se and that even when selenite Se is provided, calves may still be at risk. Sheep may also be supplemented with Se which is included in mineral mixtures and salt licks. Norwegian researchers reported no incidences of WMD in lambs and increased Se in blood and colostrum when Se fortified mineral mixtures and salt licks were offered to ewes and lambs (Overnes et al., 1985).

In addition to the use of injectable Se or the inclusion of Se in free-choice mineral mixtures, livestock producers may use an intraruminal or intrareticular bolus or pellet which provides a sustained release of Se. Judson et al. (1991) reported that the use of an intraruminal Se pellet and steel grinder increased blood Se of ewes and lambs compared with controls. However, the Se pellet and grinder system was not as effective as an injection of barium selenate at increasing blood Se. Campbell et al. (1990) used crossbred beef cows to evaluate the safety and efficacy of Se boluses and Se pellets. Both methods of Se supplementation were shown to be both safe and effective; however, blood Se of cows receiving either method of Se supplementation increased until d 119 of the study and was decreased by d 220. As in previous studies, both methods produced blood Se higher than the blood Se from unsupplemented controls. Abdelrahman and Kincaid (1995) evaluated the effects of administration of an intraruminal Se bolus on colostrum, plasma, and whole blood Se concentration of dairy cows. Those authors

reported that the Se bolus was an effective method of increasing Se in colostrum, plasma, and whole blood. Likewise, calves born to Se supplemented cows had higher Se concentrations in plasma, whole blood, and liver than calves born to cows receiving no supplemental Se. In this study, the administration of a sustained release Se bolus to cows proved to be an effective method of Se supplementation to newborn calves.

The need for Se supplementation to livestock is great as evidenced by the many benefits of supplemental Se on animal health and performance. This need is further elucidated by surveys such as reported by Dargatz and Ross (1996), which reported a relatively high percentage of beef cattle in the U.S. that were classified as Se deficient. Selenium supplementation generally adds only a negligible amount to the cost of livestock production and producers have several effective means of Se supplementation to choose from.

Absorption, Transport, Storage, and Excretion of Selenium

Ruminant animals differ from monogastric animals in their ability to absorb and/or retain Se. Wright and Bell (1966) reported retention of a dose of sodium selenite to be 29% for sheep and 77% for swine. In both sheep and swine, Se absorption occurred in the small intestine and cecum with some additional absorption in the colon for swine. No absorption of Se occurred in the rumen of sheep or the stomach of swine (Wright and Bell, 1966). These authors also reported net absorption of Se to be 36% for sheep and 86% for swine. Less absorption of Se in ruminants seems to be due to the reduction of inorganic Se to insoluble forms by rumen microorganisms (Butler and Peterson, 1961; Peterson and Spedding, 1963; Hidiroglou et al., 1968). Inorganic Se is more readily reduced within the rumen than organic forms of Se such as Se yeast. Diet also affected

Se absorption as sheep on a high concentrate diet had higher plasma Se than sheep receiving a high forage diet. (Koenig et al., 1997).

In contrast to many other minerals consumed by livestock, which use homeostasis as a primary status regulator, Se status of animals seems to have little effect on intestinal absorption. In a study utilizing rats, urinary excretion was shown as the only relevant means of Se homeostasis (Windisch and Kirchgessner, 2000) as urinary excretion is directly related to dietary level while fecal Se excretion is quite static (Burk et al., 1972). When Se absorption was regressed on Se intake of dairy cows, a strongly linear relationship was observed (Harrison and Conrad, 1984). However, Se intakes reported in that study were relatively low and ranged from 0.437 to 3.136 mg/d. Most dairy cows consume closer to 6 mg Se/d, based on supplementation in the diet of 0.3 mg/kg Se.

Absorbed Se is associated with plasma protein and transported in the blood plasma until it enters tissues (McDowell, 2003). Selenoprotein P is the plasma protein with which most Se is associated in individuals with adequate or deficient dietary Se, while most plasma Se is associated with albumin when Se intake is in excess (Xia et al., 2000).

In addition to plasma, Se is also found in muscle and glandular tissues. Generally, when ranked on a Se concentration basis, tissues follow the general order of kidney > liver > heart > skeletal muscle, regardless of species, when Se is fed at an adequate or deficient level (Comb and Combs, 1986). The kidney may be the highest in Se concentration as it is primary organ of excretion. However, when Se is fed at levels above requirement, liver surpasses kidney in terms of Se concentration (Cristaldi et al., in press).

Urine, feces, and exhalation are the primary excretion routes of Se. Amount and distribution of excreted Se within these routes are affected by chemical form of Se, total Se intake, and diet composition including antagonists (McDowell, 2003). Urine is the major excretory pathway and Se excretion via urine increases with Se status of the animal. Fecal excretion remains nearly constant and exhalation of Se becomes a major route only when Se concentrations are at a toxic level (McDowell, 2003). The amount of Se exhaled increases as dietary Se increases (McConnell and Roth, 1966) and one characteristic of animals which excrete Se via respiration is breath with a garlicky odor. Selenium excretion in ruminant animals is dependent on method of administration. When Se is provided orally, ruminants excrete more Se in feces. However, when Se is given parenterally, more Se is excreted in urine (Wright and Bell, 1966). This is supported by the concept that rumen microorganisms reduce dietary Se to insoluble forms (Butler and Peterson, 1961; Peterson and Spedding, 1963; Hidiroglou et al., 1968) and thus increase fecal excretion of unabsorbed Se.

Differences in Efficacy of Selenium due to Source

The efficacy of Se to increase blood and tissue Se concentrations in animals varies with source of Se. In general, Se is deposited in tissues and blood Se is more increased when supplemental Se is of the organic form (McDowell, 2003). The primary sources of inorganic Se are sodium selenite and sodium or barium selenate, while Se yeast and seleniferous grains and plants are the primary sources of organic Se. Sodium selenite and selenate are often added to free-choice mineral mixtures for livestock. Likewise, those two chemical forms are used in injectable Se products. Selenomethionine is the major Se compound found in grains used for livestock feeds and in Se yeast. Se-methylselenocystine is the Se compound found most abundantly in seleniferous plants,

while some inorganic Se is found in grains and plants (Whanger, 2002). In animal tissues, selenate is the major inorganic form and selenocystine is the predominant organic form. Selenomethionine is found initially when this amino acid is fed; however seleno-methionine is converted to selenocystine after some time (Whanger, 2002). With such differentiation in the sources of Se within plant and animal tissues, it seems reasonable that differences in efficacy due to form of Se administered would exist and there are numerous examples in the scientific literature to support this concept.

Goehring et al. (1984a) evaluated the effects of high dietary levels of Se from selenite or seleniferous grains on blood and tissue concentrations in swine. Those authors documented that Se from seleniferous grains increased Se in blood and tissue compared to selenite Se fed at the same level. Awadeh et al. (1998a) reported increased blood Se in crossbred beef cows consuming free-choice minerals containing 60 mg/kg Se as Se yeast compared to 60 mg/kg selenite Se. Furthermore, cows receiving free-choice minerals containing 60 mg/kg Se as Se yeast had a lower percentage protein in albumin compared to cows receiving minerals with 60 mg/kg selenite Se. Selenium from Se yeast has been documented by several groups of researchers as more effective than Se selenite or selenate at increasing blood and liver Se levels in beef cows (Pehrson et al., 1999; Valle et al., 2002; Gunter et al., 2003) and in dairy cattle (Ortman and Pehrson, 1999).

As with blood and tissue Se, milk Se has been more effectively increased by using organic Se vs inorganic Se in beef cattle, dairy cattle, and swine. Selenium yeast produced milk Se more than 100% higher than selenite or selenate Se when 3 mg of Se from each source were fed to Swedish dairy cows (Ortman and Pehrson, 1999). Hereford cows supplemented with Se yeast produced milk with markedly higher Se concentrations

than did cows receiving supplemental selenite Se in early and late lactation (Pehrson et al., 1999). In a two-yr study utilizing Florida beef cows, milk Se was consistently higher from cows receiving free-choice minerals with Se yeast compared to cows receiving Se as selenite or selenate injections (Valle et al., 2002). Also, calves suckling the cows which received the organic Se had higher Se concentrations in plasma and liver (Valle et al., 2003). Researchers at Ohio State University fed Se as Se yeast or sodium selenite at dietary levels of 0.15 and 0.30 mg/kg to gestating and lactating sows. Colostrum and subsequent milk Se concentrations were consistently at least two-fold higher from sows receiving organic Se than from sows receiving selenite Se (Mahan, 2000). Data from New Zealand indicated that the transfer of Se into cows' milk was markedly more efficient, up to three-fold more, with selenized yeast than with sodium selenate (Knowles et al., 1999).

The effectiveness of different sources of Se for supplementation continues to be evaluated even though Se has been recognized as nutritionally essential since the late 1950s. Selenium provided by different supplementation methods and from different sources leads to different physiological responses in the animals that serve mankind. With evidence of an increasing ability to manipulate the Se content of milk and animal tissues which are commonly consumed by humans, it seems to be possible to supplement Se to humans through method and source of Se supplementation to livestock. Givens et al. (2004) reported a decline in Se intake by the people of Great Britain. After conducting an experiment which validated previous findings that the milk of dairy cows could be increased by feeding an organic Se source, those authors further explored the idea of increasing human consumption of Se by altering the Se content of foods. It seems

that a worthy challenge exists for animal scientists and food scientists to work collaboratively to identify effective programs for administration of Se to dairy and food animals so that the milk and meat subsequently produced can be more nutritious for humankind.

Selenium Toxicosis

Selenium deficiencies are prevalent in many parts of the world (McDowell, 2003) and the benefits of Se supplementation continue to be elucidated. However, it seems that Se is still most often implicated as an element which is toxic to livestock. This belief most likely stems from diary-style documentation, observations, and research findings beginning as early as 1295 when Marco Polo described an agent in plants which when eaten by horses caused their hooves to fall off (Komroff, 1926). Six hundred years later similar afflictions began to be described in the Great Plains region of the United States. In 1856, a U.S. Army surgeon reported the occurrence of a disease, fatal to U.S. Cavalry horses, similar to the affliction described by Marco Polo (Madison, 1860). The horses in the Nebraska Territory near Fort Randall lost hair and had debilitating conditions of the hoof. By the 1890s, farmers and stockmen who settled in northern Nebraska and South Dakota observed similar conditions in livestock (Moxon and Rhian, 1943). Selenium toxicity in livestock and laboratory animals has been reported from the 1930s to the present day. Some reports were observations of animals receiving seleniferous grains or grazing seleniferous plants (Franke, 1934; Franke and Potter, 1935; Moxon, 1937). Other researchers have intentionally induced or attempted to induce Se toxicities, while several reports of Se toxicity are a result of accidental overdosing with injectable Se.

Rosenfeld and Beath (1964) suggested that Se poisoning in livestock occurs in three distinct phases: acute toxicity and the two phases of chronic toxicity, alkali disease

and blind staggers. Acute Se toxicity can be caused by ingesting a large amount of supplemental Se, overdosing with parenteral Se, or by ingesting a large amount of seleniferous plants. Certain plants, mostly species of Astragalus, may contain up to 10,000 mg/kg Se and cereal crops, grasses, and other forages may contain up to 50 mg/kg Se (Aitken, 2001). Fatalities of sheep, cattle, and hogs have been reported in regions known to grow seleniferous plants (National Academy of Sciences [NAS], 1983), with some deaths occurring within 24 h (Rosenfeld and Beath, 1964). Clinical signs of acute Se toxicity may include elevated body temperature, labored breathing, diarrhea, and often death. Alkali disease and blind staggers types of Se toxicosis occur with more time and involve feedstuffs containing less Se. Clinical signs of chronic Se toxicity include anorexia, apathy, diarrhea, weight loss, hair loss, and hoof malformations (Glenn et al., 1964a). Animals in the blind staggers phase of Se toxicosis may wander, stumble, and lack appetite initially and then become somewhat paralyzed and almost blind in the later stage. The later stage appears suddenly and death usually occurs within hours (Rosenfeld and Beath, 1964). Chronic forms of selenosis have been induced by feeding grains containing 5 to 40 mg/kg Se (Schoening, 1936; Rosenfeld and Beath, 1964).

Glenn et al. (1964a) induced death in ewes after oral dosing of up to 50 mg/d of Se as sodium selenate for 93 d and concluded that minimum toxic oral dose of Se as selenate depended on susceptibility, level of Se administered, and duration of administration. Those authors later reported liver Se concentrations of up to 29 mg/kg in experimentally poisoned ewes (Glenn et al., 1964c). Evaluation of the tissues of the ewes in the previous study showed that most tissue damage in Se toxicosis is confined to the heart (Glenn et al., 1964b). No kidney damage and few instances of liver damage were reported.

Blodgett and Bevill (1987) induced death in sheep by feeding 0.7 to 1.0 mg Se/kg BW as selenite in as little as 6.75 h. Liver Se concentrations of more than 17 mg/kg and whole blood Se of 2.7 mg/L were reported. After receiving an oral 5 mg selenite Se/kg BW, lambs died within 6 h and when the same dosage was given as an injection, lambs lived up to 60 h (Smyth et al., 1990). After evaluating the major organs, those authors concluded that the heart is most damaged in a case of Se toxicosis as the heart has great affinity for Se especially in lethal doses. Caravaggi et al., (1970) injected Merino lambs with 0.425 to 0.500 mg Se/kg BW, induced death, and determined the LD_{50} for lambs to be 0.455 mg Se/kg BW. Twenty lambs received 10 mg of selenite Se orally in an attempt to prevent WMD. Of those 20, seven died within 16 h, eight developed diarrhea but recovered, and five lambs were apparently unaffected (Marrow, 1968). Cristaldi et al. (2004) fed up to 10 mg/kg dietary Se as selenite to growing wether sheep and reported no signs of Se toxicity. Those authors reported whole blood Se concentrations of up to 1.2 mg/L, wool Se of 2.5 mg/kg, and liver Se concentrations of nearly 15 mg/kg Se on a dry basis and no evidence of Se toxicity from histopathological evaluation.

Selenium toxicity studies have also been conducted using swine. Goehring et al. (1984b) fed young pigs up to 20 mg/kg Se as sodium selenite for 5 wk. No pigs on the study died; however, feed intake and growth rate decreased as dietary Se concentration increased. Whole blood Se concentrations of up to 3.5 mg/L and hair Se of more than 11 mg/kg were observed. Organic and inorganic Se was included in the diet of growing pigs at levels of up to 20 mg/kg for 12 wk (Kim and Mahan, 2001). Feed intakes of those pigs declined as Se level increased and daily gains were decreased when Se was fed at more than 5 mg/kg, and inorganic Se had a more detrimental effect on performance than did

organic Se. Those authors reported plasma Se concentrations of more than 3.3 mg/L, liver Se of more than 17 mg/kg, and hoof Se of more than 28 mg/kg and no deaths regardless of dietary Se level. The authors concluded that the higher retention of organic Se in tissues and blood cells may effectively reduce the amount of Se available to cause Se toxicosis.

Tolerance of Se as selenite or selenomethionine was evaluated using yearling steers in a 4 mo study (O'Toole and Raisbeck, 1995). Those authors observed the highest incidence of hoof lesions in steers fed organic Se at a rate of 0.80 mg/kg BW. Likewise, it was shown that the steer with the most severe hoof lesions also had the highest Se concentrations in hair, liver and kidney. The findings of that study indicated that dietary exposure of 0.8 mg Se/kg BW, in either form, for 4 mo produces subclinical to clinical signs of Se toxicosis. The authors concluded that selenomethionine is more likely to cause alkali disease than sodium selenite. Holstein cows were fed inorganic Se up to 100 mg/d and whole blood and liver Se concentrations of up to 4.9 mg/L and 15 mg/kg DM, respectively, were reported (Ellis et al., 1997). It was concluded that dairy cattle could tolerate Se intakes of up to 100 mg/d for several wk without suffering adverse affects.

It seems logical, based on previous findings, that the minimum lethal dosages and maximum tolerable levels of Se are variable and may be affected by various factors such as Se source (organic or inorganic), diet composition, method of Se supplementation, and Se status of the animal. The current maximum tolerable level for dietary Se in domestic animals is 2 mg/kg (NRC, 1980). This estimate does not consider differences in species, source of Se, or duration of exposure. Early reports of toxicities are likely reasons for the conservative estimate of maximum tolerable level and the notion that the range between

optimal and toxic level of Se is narrow. Surely, further studies which are long in duration and use high dietary levels and different sources of Se are necessary to better estimate the tolerance of Se for dairy and food animals.

CHAPTER 3
TOLERANCE OF INORGANIC SELENIUM IN RANGE-TYPE EWES DURING GESTATION AND LACTATION

Introduction

Since its discovery by Berzelius in 1817, Se has had a rich and colorful history in animal agriculture. Though much of the world is troubled with Se deficiencies (McDowell, 2003), Se toxicities present a greater problem to control. In 1957, Se was established as an essential nutrient and the benefits of Se supplementation to livestock continue to be elucidated. Current estimates put the maximum tolerable level of Se at 2 mg/kg for the major livestock species (NRC, 1980) and no differentiation exists for tolerable levels between ruminants and monogastric animals. However, the work of Butler and Peterson (1961) and Hidiroglou et al. (1968) suggests that inorganic Se (e.g., sodium selenite) may be reduced to insoluble selenide by microorganisms in the rumen, thus reducing overall absorption of Se by ruminant animals. Wright and Bell (1966) reported that swine retained 77% of an oral dose of inorganic Se, which is nearly three-fold the retention by sheep. Selenium toxicities have been often produced by researchers in ruminants, but they are generally induced by Se injections (Marrow, 1968; Caravaggi et al., 1970; Shortridge et al., 1971) or by feeding Se above maximum tolerable levels (5 to196 ppm) to monogastric animals (Franke and Potter, 1935; Miller and Schoening, 1938; Kim and Mahan, 2001). More recently, Cristaldi et al. (2004) demonstrated that wether sheep did not display signs of Se toxicosis after receiving up to 10 mg/kg dietary Se for one yr. Based on these and other previous findings, it seems that the current maximum tolerable level of Se for ruminants is underestimated. Most Se toxicity

research in ruminants has been documented in lambs or wethers. Controlled experiments using ewes during stresses of production (e.g., gestation and lactation) are lacking in the scientific literature. The objective of this long-term (72 wk) study were to evaluate and compare effects of feeding Se as sodium selenite at supranutritional levels on ewe serum, blood, wool, and tissue Se concentrations during two lambing periods and to determine maximum tolerable level of Se.

Materials and Methods

All animal procedures were conducted within the guidelines of and approved by the University of Florida Institutional Animal Care and Use Committee. This experiment utilizing ewes during two lambings was conducted from December 18, 2001 to May 5, 2003 at the University of Florida Sheep Nutrition Unit located in North Central Florida. Forty-one, four-yr-old, Rambouillet ewes, that originated from a single range flock in Texas and had been pasture exposed to rams during October and early November 2001 (average 57 d gestation), were weighed (57.4 ± 5.7 kg) and administered 2-ml ivermectin dewormer s.c. (Ivomec; Merial Ltd., Iselin, NJ). Ewes were randomly assigned to one of six dietary treatments for a 72-wk study. Six dietary treatments were 0.2, 4, 8, 12, 16, or 20 mg/kg Se as sodium selenite (as-fed basis) added to a corn-soybean meal basal diet (Table 3-1). The basal diet was formulated to meet animal requirements for protein, energy as TDN, vitamins, and minerals for this class of sheep (NRC, 1985). Animal numbers per treatment were six for 0.2 (control) and seven each for 4, 8, 12, 16, and 20 mg/kg added Se treatments. Ewes were housed by treatment group in covered wooden pens (53.5 m^2) with earth floors and ad libitum water.

Diets were fed at 909 $g \cdot ewe^{-1} \cdot d^{-1}$ from d 0 until lambing began, increased to 1000 $g \cdot ewe^{-1} \cdot d^{-1}$ during lambing, and again increased to 1135 $g \cdot ewe^{-1} \cdot d^{-1}$ during lactation. Ewes received 909 $g \cdot ewe^{-1} \cdot d^{-1}$ of their respective diets after the first lamb crop was weaned. On August 15, 2002, ewes were pen exposed to rams for 35 d. Diets were offered at the same increments during the second lambing and lactation as during the first. Diets were sampled every 28 d, ground (1 mm), and frozen at 0°C until analysis.

Ewe BW was recorded on d 0 and for every four wk thereafter, for the remainder of the study. A 10-mL blood sample for serum analysis was collected using an 18-gauge needle into a vacutainer tube with no additive (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) every four wk, via jugular venipuncture, allowed to stand for 20 min, centrifuged at 700 x g for 25 min, and serum stored frozen at 0°C until Se analysis. Starting at wk 12, an additional 10-mL blood sample was collected into a heparinized vacutainer tube (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). This additional 10-mL sample was collected every 12 wk for the remainder of the experiment and stored frozen at 0°C as whole blood until analysis.

The wool around the jugular was shorn initially and regrowth was collected beginning at wk 12 and every 12 wk thereafter. The collected wool was washed with a commercial hair shampoo (Alberto VO5; Alberto-Culver Co., Melrose Park, IL), to remove oil and dirt, rinsed well with deionized water, dried, stored at room temperature, and later analyzed for Se concentration.

At the termination of the experiment (wk 72), ewes were slaughtered following approved USDA procedures at the University of Florida Meats Laboratory. An additional 10-mL sample of blood was collected using an 18-gauge needle into a

vacutainer, centrifuged at 700 x g for 25 min, and serum frozen at 0°C for analysis of albumin and the following enzymes: alkaline phosphatase (Alk Phos), alanine transaminase (ALT), aspartate transaminase (AST), creatinine phosphokinase (CK), and gamma glutamyl transferase (GGT). Albumin and the enzymes were analyzed in order to determine possible tissue breakdown as a result of Se toxicosis.

Samples of brain, diaphragm, heart, hoof tip, kidney, liver, and psoas major muscle were collected, and frozen (0°) until analyzed for Se. Sections (1 cm^3) of liver, heart, kidney, diaphragm, and psoas major muscle from all animals were placed in 10% neutral-buffered formaldehyde for subsequent microscopic evaluation for evidence of Se toxicosis.

For histopathological evaluation, the tissue samples fixed in buffered formalin were embedded in paraffin and sectioned at 6 microns. All sections were stained with hematoxylin and eosin, and examined under a light microscope (10X, 20X, and 40X). Serum albumin, Alk Phos, ALT, AST, CK, GGT were evaluated on a Hitachi 911 analyzer with reagents from Sigma (Sigma Chemical Co., St. Louis, Mo.). These procedures were established by the Veterinary Medical Teaching Hospital at the University of Florida.

Serum, whole blood, wool, tissue, and feed samples were analyzed for Se concentration using a fluorometric method described by Whetter and Ullrey (1978). To help ensure reliability of the analytical method, a certified standard (National Bureau of Standards Bovine Liver SRM-1577a; U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) was frequently analyzed.

Brain, diaphragm, heart, hoof tip, kidney, liver, and psoas major muscle Se data were analyzed for effects of treatment using PROC GLM in SAS (SAS for Windows 8e; SAS Inst., Inc., Cary, NC) in a completely randomized design. Pre-planned orthogonal contrast statements were used to compare means as described by Littell et al. (1998; 2000). PROC MIXED of SAS was used to analyze effects of treatment, time, and the interaction of treatment × time on BW, serum Se, whole blood Se, and wool Se as repeated measures with a spatial power covariance structure with respect to d and a subplot of animal nested within treatment. Pre-planned orthogonal contrast statements were written to determine differences in means at different sampling intervals. Means were separated at $P < 0.05$ and regression analysis was used to determine relationships between dietary Se and Se concentration of various tissues.

Results and Discussion

Performance

Ewe BW was not affected by dietary Se level ($P = 0.69$) or dietary Se level × time interaction ($P = 0.56$). However, time did affect BW ($P < 0.001$). Initial BW was 57.4 ± 5.7 kg and BW at the termination of the experiment was 61.2 ± 15.1 kg. These findings agree with previous studies in ruminants. Supplemental selenium fed up to 0.4 mg/kg which is above requirement but below maximum tolerable level had no effect on rate of gain in feedlot steers (Perry et al., 1976) and BW gains in wether sheep, fed sodium selenite up to 10 mg/kg, was unaffected by dietary Se level (Cristaldi et al., in press). Glenn et al. (1964a) also reported no effect of dietary Se on BW when sodium selenate was fed to ewes as a single oral dose of up to 50 mg/d. The ewes utilized by those authors were very similar in breed type and BW to the animals used in the present study.

Effect of time on ewe BW can be explained by changes in BW associated with gestation and lactation over two lambings during the study.

Ten of 41 ewes died over the course of this 72-wk study. Gross necropsies were performed on eight ewes following death. Tissues from two ewes were too severely decomposed to allow for evaluation for pathological changes. Necropsy of eight ewes cited causes of death as lymphadenitis associated with injury (two ewes), endoparasitism (two ewes), ketosis (three ewes) and pneumonia (one ewe). Pathological evidence of Se toxicosis was not found in any ewe that died before the termination of the experiment.

In the first yr, 53 lambs were born over 20 d from March 9, 2002 to March 28, 2002. Fifty-two lambs were born alive and unassisted (Table 3-2). One lamb was very large (8 kg) and died shortly after a difficult birth. The lambs born in yr one represent a 129% lamb crop when calculated as lambs born alive per ewe exposed. In the second yr, 36 lambs were born over 34 d from January 17, 2003 to February 20, 2003 (Table 3-2). All lambs were born alive and unassisted. Thirty-six lambs in yr two represent a 109% lamb crop as only 33 ewes were exposed in the second yr. Number of lambs born per ewe did not affect serum Se concentration ($P > 0.54$) of ewes receiving any level of dietary Se. Glenn et al. (1964a), who fed higher levels of dietary Se than in the present experiment, did not observe an effect of dietary Se level on reproduction in 2-yr-old range ewes. Those researchers observed a similar number of pregnancies in each treatment group and no malformations in lambs. In contrast, Rosenfeld and Beath (1947) observed lamb deformities in a field study and attributed the anomalies to excess Se in ewe diets. However, seleniferous plants were the Se source, rather than inorganic sources used in the present experiment. Furthermore, in a grazing situation, it is possible that

lamb deformities were due to toxic elements other than Se. In both yr of our study, all lambs were born free of congenital deformities, but the number of pregnancies were lowest in ewes receiving 16 mg/kg dietary Se, but not 20 mg/kg. However, breeding soundness evaluations were not performed on ewes or rams used in this study and thus, to incriminate or exclude dietary Se level as a detriment to ewe reproduction would be observational.

Blood

Serum Se concentrations from wk 4, 8, and 12 were analyzed together and will be referred to throughout the results and discussion as late gestation yr 1. Lactation yr 1 includes serum Se concentrations from wk 12, 16, 20, and 24. Week 12 is included in both late gestation and lactation for yr 1 as some ewes were lactating and some remained in late gestation when wk 12 sampling occurred. Weeks 28, 32, 36, 40, and 48 compose the dry, rebreeding period. Late gestation in yr 2 includes serum Se measurements from wk 52, 56, and 60. Lactation in yr 2 includes wk 60, 64, 68, and 72. Similar to yr 1, one sampling date (wk 60) was common to both late gestation and lactation and was included in both periods.

During all stages of lamb production, serum Se increased in a linear fashion ($P < 0.001$) as dietary Se level increased (Table 3-3). This agrees with previous Se toxicity research as Se concentrations in serum of wether sheep (Cristaldi et al., in press) also increased linearly as dietary selenite Se was increased. All ewes had similar ($P > 0.82$) serum Se at the initiation of this experiment. Initial serum Se ranged from 90 to 120 μg/L, which is below the normal range (120 to 180 μg/L) for adult sheep (Aitken, 2001). A cubic response within treatment ($P = 0.02$) was observed in serum Se across the stages of production (time) from wk four to wk 72. Ewe serum Se, in general, was higher

during the dry, rebreeding stage. One plausible explanation for this is the lack of placenta, fetal tissue, and milk for deposition and excretion of Se. During late gestation in yr 1, dietary Se level affected serum Se concentration ($P < 0.001$), ewes receiving 8, 12, 16, and 20 mg/kg Se all had higher ($P < 0.05$) serum Se than did controls. Likewise, ewes receiving 16 or 20 mg/kg Se had serum Se higher ($P < 0.05$) than ewes receiving 4 mg/kg Se. During lactation in yr 1, ewes receiving 16 and 20 mg/kg Se were similar ($P = 0.32$) and both groups were higher ($P < 0.05$) than controls and ewes receiving 4 and 8 mg/kg Se in serum Se concentration. During the 20 wk that ewes were not lactating and were either open or rebreeding, ewes receiving 16 and 20 mg/kg dietary Se had similar ($P = 0.44$) serum Se which was higher ($P < 0.05$) than from all other treatments. Ewes receiving the intermediate levels of Se (8 and 12 mg/kg) had similar serum Se ($P = 0.31$) which was higher ($P < 0.05$) than from controls and ewes receiving 4 mg/kg Se. Ewes in late gestation during yr 2 generally produced numerically higher serum Se than in late gestation the previous yr. Ewes receiving 20 mg/kg Se had serum Se which was similar ($P = 0.69$) only to serum Se from ewes receiving 16 mg/kg Se and higher ($P < 0.05$) than all other treatments. Ewes receiving 16 mg/kg Se produced serum Se which tended ($P = 0.07$) to be higher that serum Se from ewes receiving 12 mg/kg Se and was higher ($P < 0.05$) than serum Se from controls and ewes receiving 4 or 8 mg/kg Se. During lactation in yr 2, ewes receiving 20 mg/kg Se had higher ($P < 0.05$) serum Se than serum Se from all other treatments. Serum Se from ewes receiving 8, 12, or 16 mg/kg Se was similar ($P > 0.20$) and only serum Se from ewes receiving 4 mg/kg dietary Se was similar ($P = 0.21$) to controls. Throughout the experiment, serum Se concentrations in these ewes remained below 1500 µg/L, which is described as a toxic level in horses (Aitken, 2001) and were at

most 37% of a reported toxic level (3700 μg/L) in swine (Aitken, 2001). Caravaggi et al. (1970) established an LD_{50} for sheep at 455 μg/kg BW. When our data are described on a μg/kg BW basis using the highest dietary concentration (20 mg/kg), highest daily intake (1135 g/d), and average ewe BW (60 kg), our ewes were consuming, at maximum, 378 μg/kg BW. This is 17% less than the LD_{50} for sheep as previously described. The ewes in the present study were mature and maintained healthy ruminal function throughout the study. This is contrasted with the unweaned lambs used by Caravaggi et al. (1970). Those lambs may have received Se via i.m. injection. Administration of Se parenterally disallows the reduction of selenite Se to insoluble selenide via ruminal microorganisms as described by (Whanger et al., 1968). This would suggest that the LD_{50} for sheep could be considerably higher than previously thought. Glenn et al. (1964a) fed sodium selenate at high levels to range ewes that were similar in BW to ewes on the present study. Those researchers did not induce death by Se toxicosis with daily oral doses less than 25 mg Se/ewe. Of the 17 deaths reported in their experiment, only one was induced with a daily dose of 25 mg Se/ewe. Eight deaths were induced with a daily dose 37.5 mg Se/ewe and eight deaths were induced with a daily dose 50 mg Se/ewe. Those deaths were not by acute Se toxicosis. The ewes received experimental Se doses for at least 80 d before death by Se toxicosis was induced. In the same experiment, Glenn et al. (1964a) further suggested an average minimum toxic level of Se for adult sheep to be 0.825 mg/kg BW when fed for 100 d. Using this estimate, the minimum toxic level of Se for ewes of the size used in our study would be 50.3 mg/d. Selenium consumption, at the highest dietary level of 20 mg/kg, never reached even 50% of that previously reported level throughout our study. Also, Blodgett and Bevill (1987) reported an LD_{50} for sheep, using sodium

selenite via i.m. injection, at 0.7 mg/kg BW. Other researchers (Rosenfeld and Beath, 1946) reported death in sheep with less Se (30 mg/d); however, the Se maximum intake level used in our study was approximately 25% less. It is important to note that we used sodium selenite as our Se source whereas previous research (Rosenfeld and Beath, 1946; Caravaggi et al., 1970) used sodium selenate as the source of additional Se. Henry et al. (1988) reported a higher relative bioavailability for selenate than selenite. This suggests the possibility of a higher tolerance for sodium selenite vs selenate.

Whole blood Se was measured at wk 12, 24, 36, 48, 60, and 72 (Table 3-4). Dietary Se level, time, and dietary Se level × time affected ($P < 0.05$) ewe whole blood Se. Whole blood Se increased linearly ($P < 0.001$) as dietary Se increased. Response of whole blood Se from all treatments over time was cubic ($P < 0.01$) which agrees with the time response of serum Se. Maas et al. (1992) reported a strong correlation (0.88) for whole blood Se and serum Se. Our data support this relationship, as serum Se and whole blood Se responded to dietary Se level in a similar fashion. The cubic response of whole blood Se over time may be attributed to ewes having no fetal tissue and producing no milk to use as a route of excretion during the dry, rebreeding period, which encompassed the midpoint of this study. Each dietary Se level was evaluated individually over time and control and 8 mg/kg neither increased nor decreased with time ($P > 0.20$). Whole blood Se from ewes receiving 4 mg/kg Se responded cubically ($P = 0.019$) and 16 mg/kg dietary Se tended ($P = 0.07$) tended to respond cubically. Whole blood Se concentration changed more sporadically over time in ewes receiving 12 or 20 mg/kg dietary Se and each treatment produced a fifth degree polynomial ($P < 0.05$). At wk 12, ewes in all treatment groups had higher ($P < 0.05$) whole blood Se than did controls. Ewes receiving

20 mg/kg Se had higher whole blood Se than controls and ewes receiving 4, 8, or 16 mg/kg Se and tended be higher ($P = 0.13$) in whole blood Se than ewes receiving 12 mg/kg dietary Se. At wk 24, ewes receiving 20 mg/kg Se had higher whole blood Se than ewes from all other treatment groups and only ewes receiving 4 mg/kg Se had whole blood Se similar to controls. At wk 36, ewes receiving 12, 16, and 20 mg/kg Se had similar ($P > 0.05$) whole blood Se and again, only ewes receiving 4 mg/kg Se had whole blood Se similar to controls. At wk 48, whole blood Se concentrations from ewes receiving 16 and 20 mg/kg Se were similar ($P > 0.10$) and higher than ($P < 0.05$) from ewes on all other treatments. Ewes receiving 8 and 12 mg/kg Se had similar ($P = 0.88$) whole blood Se concentrations which were higher ($P < 0.05$) than those from controls and ewes receiving 4 mg/kg Se. Only whole blood Se from ewes receiving 4 mg/kg Se was similar ($P = 0.16$) to controls at wk 48. Whole blood Se concentrations at wk 60 followed a pattern similar to wk 48, in terms of differences among treatments. At wk 72, whole blood Se from four of six dietary levels had numerically decreased from wk 60. Whole blood from ewes receiving 20 mg/kg Se was higher ($P < 0.05$) in Se concentration than in ewes from all other treatments. Ewes receiving 4, 8, 12, and 16 mg/kg dietary Se produced similar ($P > 0.10$) whole blood Se and only ewes receiving 12 mg/kg Se had higher ($P < 0.05$) whole blood Se than did controls. Cristaldi et al. (2004) also reported a linear increase in whole blood Se as dietary Se was increased. Likewise, those authors noted differences in treatment means over controls as dietary Se levels were increased up to 10 mg/kg. Increased whole blood Se concentrations were reported in dairy cows as their salt-based mineral mixtures were increased from 20 mg/kg to 120 mg/kg selenite Se

(Awadeh et al., 1998a). Whole blood Se increased linearly in young swine as dietary Se was fed up to 20 mg/kg (Goehring et al., 1984b).

Wool

Selenium concentration in new growth wool was measured at wk 12, 24, 36, 48, 60, and 72 (Table 3-5). Dietary Se level, time, and dietary Se level × time affected ($P < 0.001$) wool Se. Wool Se increased linearly ($P < 0.001$) as dietary Se increased. Response of wool Se over time was quadratic ($P < 0.001$) and time response for each dietary Se level was evaluated individually. Wool Se from controls and ewes receiving 8, 12, and 16 mg/kg dietary Se responded quadratically ($P < 0.03$) from wk 12 to wk 72. Wool Se from ewes receiving 4 mg/kg Se responded cubically ($P < 0.05$) and wool Se from ewes receiving 20 mg/kg Se increased linearly ($P < 0.01$) over time. Increased Se in hair has been reported in other livestock species. Kim and Mahan (2001) observed a linear response in the hair of pigs as Se in their diet was increased. Goehring et al. (1984b) reported a quadratic response in the hair of swine as dietary Se as sodium selenite was increased up to 20 mg/kg. Likewise, Perry et al. (1976) reported increased Se in the hair of feedlot steers as dietary selenite Se was increased. Cristaldi et al. (2004) reported a linear increase in the wool of growing sheep as dietary Se was increased and also observed differences in wool Se of wethers receiving 6, 8, or 10 mg/kg Se vs controls. These authors did not report a significant treatment × time interaction. However, wool Se in the present study was affected by time and the interaction of treatment × time as wool Se increased and then seemed to reach a plateau around wk 48. Kim and Mahan (2001) and Cristaldi et al. (2004) used 10 mg/kg Se as the highest dietary level and reported linear responses in hair and wool. However, with 20 mg/kg as the highest dietary level, the quadratic responses observed by Goehring et al. (1984b) and

in our study suggest that Se in wool and hair does not continue to increase linearly as dietary Se is increased above 10 mg/kg. At wk 12, only ewes receiving 20 mg/kg Se had wool Se higher ($P < 0.05$) than controls, however wool Se from ewes receiving 12 and 16 mg/kg Se tended ($P < 0.15$) to be higher than from controls. At wk 24, wool Se from ewes receiving 16 or 20 mg/kg Se was higher than from controls and ewes receiving 4 mg/kg Se. Wool Se from ewes receiving 8 or 12 mg/kg Se tended ($P < 0.07$) to be higher than wool Se from ewes receiving 4 mg/kg Se. At wk 36, wool Se from ewes on all treatment groups was higher ($P < 0.05$) than from controls, and Se concentrations in wool from ewes receiving 16 mg/kg Se were higher ($P < 0.05$) than wool Se from ewes receiving 4 or 12 mg/kg dietary Se. Wool Se concentrations from ewes on all treatment groups were similar ($P > 0.15$) and higher ($P < 0.05$) than wool Se from controls at wk 48. At wk 60, again, wool Se concentrations from ewes on all treatment groups were higher ($P < 0.05$) than wool Se from controls. At the termination of the experiment, wool Se from ewes receiving 20 mg/kg Se was higher than from ewes on all other treatments and ewes from all treatment groups produced higher ($P < 0.05$) wool Se than did controls. Some wool loss was observed in two ewes receiving 20 mg/kg dietary Se during lactation in yr one. However, after lambs were weaned and lactation had ceased, both ewes regrew a full fleece.

Tissues

Selenium concentrations in all tissues were affected ($P < 0.001$) by dietary Se level. Selenium concentrations in brain ranged from 1.90 to 6.45 mg/kg DM and increased linearly ($P < 0.05$) as dietary Se increased (Figure 3-1). Regressing brain Se (mg/kg DM) on dietary Se concentration (mg/kg) produced the following relationship:

Brain Se = 1.89 + 1.56 Dietary Se ($r^2 = 0.52$; $P < 0.05$).

Ewes consuming 12 or 20 mg/kg Se had higher ($P < 0.05$) brain Se than controls and ewes consuming 20 mg/kg Se had higher ($P < 0.05$) brain Se than ewes consuming Se at all levels except 12 mg/kg.

Diaphragm Se ranged from 1.27 to 4.01 mg/kg DM increased ($P < 0.05$) in a linear manner as dietary Se was increased. (Figure 3-1). Regressing diaphragm Se (mg/kg DM) on dietary Se concentration (mg/kg) produced the following relationship:

Diaphragm Se = 1.27 + 1.33 Dietary Se ($r^2 = 0.63$; $P < 0.05$).

Ewes receiving 20 mg/kg Se had higher ($P < 0.05$) diaphragm Se than ewes receiving all other treatments and only ewes receiving 12 or 20 mg/kg Se had higher diaphragm Se than controls ($P < 0.005$).

Heart tissue Se (Figure 3-1) ranged from 1.83 to 6.24 mg/kg DM and increased in a linear fashion ($P < 0.001$). Regressing heart Se (mg/kg DM) on dietary Se concentration (mg/kg) produced the following relationship:

Heart Se = 1.83 + 1.99 Dietary Se ($r^2 = 0.70$; $P < 0.05$).

Ewes receiving 12, 16, and 20 mg/kg Se had higher ($P < 0.05$) heart Se than controls and ewes receiving 4 and 8 mg/kg Se tended to have higher ($P < 0.12$) heart Se than controls. Heart Se concentrations from ewes receiving 20 mg/kg Se were higher ($P < 0.05$) than those from ewes receiving all other dietary Se levels.

Selenium concentration in hoof ranged from 0.93 to 7.68 mg/kg DM and increased cubically as dietary Se increased (Figure 3-2). Regressing hoof Se (mg/kg DM) on dietary Se concentration (mg/kg) produced the following relationship:

Hoof Se = 0.93 + 1.95 Dietary Se – 0.49 Dietary Se^2 + 0.06 Dietary Se^3 ($r^2 = 0.60$; $P < 0.05$).

Ewes receiving 16 and 20 mg/kg Se had higher hoof Se ($P < 0.05$) than controls. Likewise, ewes receiving 20 mg/kg Se had higher hoof Se ($P < 0.05$) than ewes receiving 4, 8, and 12 mg/kg dietary Se.

Selenium concentrations in psoas major muscle (i.e. tenderloin), a muscle commonly consumed by humans, ranged from 0.60 to 3.66 mg/kg DM and increased linearly as dietary Se increased (Figure 3-2). Regressing psoas major muscle Se (mg/kg DM) on dietary Se concentration (mg/kg) produced the following relationship:

Psoas major muscle Se = 0.59 + 1.42 Dietary Se ($r^2 = 0.62$; $P < 0.05$).

Selenium concentrations in psoas major muscle from controls were lower ($P < 0.05$) than from ewes receiving 4, 12, 16, and 20 mg/kg Se and tended to be lower ($P = 0.06$) than psoas major muscle Se concentrations from ewes receiving 8 mg/kg Se. Ewes receiving 20 mg/kg Se had higher ($P < 0.05$) psoas major muscle Se than ewes receiving all other Se levels.

Kidney Se ranged from 5.18 to 31.61 mg/kg DM and responded to increased dietary Se in a cubic fashion (Figure 3-2). Regressing kidney Se (mg/kg DM) on dietary Se concentration (mg/kg) produced the following relationship:

Kidney Se = 5.18 + 6.64 Dietary Se – 2.28 Dietary Se^2 + 0.32 Dietary Se^3 ($r^2 = 0.62$; $P < 0.05$).

Ewes receiving 20 mg/kg Se had higher ($P < 0.01$) kidney Se than ewes from all other treatment groups. Ewes receiving 12mg/kg Se tended ($P = 0.09$) to have higher ($P < 0.01$) kidney Se than controls.

Liver Se concentration ranged from 4.20 to 230.36 mg/kg DM and responded quadratically as dietary Se level increased (Figure 3-3.) Regressing liver Se (mg/kg DM) on dietary Se concentration (mg/kg) produced the following relationship:

Liver Se = 4.19 + 26.59 Dietary Se – 9.31 Dietary Se^2 ($r^2 = 0.66$; $P < 0.01$). Ewes receiving 20 mg/kg dietary Se had higher ($P < 0.05$) liver Se than ewes from all other treatments. No other differences ($P > 0.05$) existed among controls and Se treatment groups.

Linear increases in the Se concentration of loin, liver, kidney and hoof were reported in swine (Kim and Mahan, 2001) and sheep (Cristaldi et al., in press). Likewise, Echevarria et al. (1988) reported linear responses of sheep liver, kidney, heart, and muscle to dietary Se as sodium selenite Se. In our study, loin, diaphragm, heart, and brain responded linearly, where kidney and hoof responded cubically and liver responded quadratically. These higher degree polynomials may be due to changes in metabolism of Se as dietary Se concentration approaches 20 mg/kg. Most previous research used 10 mg/kg Se as the highest dietary concentration.

Enzymes and Histopathology

Serum for evaluation of albumin and enzyme activities was collected at wk 72 along with samples of brain, diaphragm, heart, hoof tip, kidney, psoas major muscle, and liver for histopathological evaluation. Concentrations of albumin and activities of Alk phos, ALT, GGT, AST, and CK in serum were in or below the normal range for adult sheep (Table 3-6). In instances of Se toxicosis, the activities of these enzymes would have been increased due to tissue necrosis. Our observations agree with those reported by Cristaldi et al. (2004) as albumin and enzyme activities in wether sheep after receiving up to 10 mg/kg Se were in the normal ranges.

Most of the tissues collected at slaughter were free from pathological changes. The findings of lymphocytes in the portal triads were deemed to be a background finding and insignificant. Likewise, the findings of lymphocytic foci in the heart tissue were determined to be associated with sarcocystic parasites. Mineral precipitations were observed in kidney tissue of some ewes and are incidental, background findings. Contraction bands present in the diaphragm and psoas major muscle were a result of stunning during humane slaughter. Adipose tissue was present in the heart and psoas major muscle, which is an indication of adequate nutrition. Hepatic lipidosis was diagnosed in four ewes. Two cases (one severe, one moderate) were diagnosed in ewes receiving 16 mg/kg dietary Se. In the moderate case, there was also evidence of bile retention. Neither of these ewes lambed in either yr. This would indicate that the hepatic lipidosis could be treatment related rather than due to metabolic changes associated with gestation, parturition, and lactation. One ewe receiving 12 mg/kg Se and one ewe receiving 4 mg/kg were diagnosed with mild hepatic lipidosis, however, both ewes lambed in both yr. Thus, the hepatic lipidosis was likely due to metabolic changes associated with lamb production. No evidence of significant pathological changes was observed in ewes receiving 20 mg/kg dietary Se, which was the highest Se level used in this study. Cristaldi et al. (2004) found no abnormalities after microscopic evaluation of heart, liver, kidney, diaphragm, and muscle from wethers consuming up 10 mg/kg Se for one yr. Likewise, only one instance of abnormal pathology was observed in ewes consuming less than 10 mg/kg Se on our study. Furthermore, our study was approximately 40% longer in duration, utilized treatments of up to 100% more Se, and introduced stresses of production, all of which should have helped to induce Se toxicosis

and thus, the finding of abnormal organ pathology. However, abnormal pathological findings were few and did not follow a pattern with respect to dietary level which would be indicative of Se toxicosis.

No clinical signs of Se toxicosis such as abnormal hoof growth or loss of wool were observed in ewes receiving > 16 mg/kg Se. However, some excessive hoof growth was observed after approximately one yr in ewes receiving 16 and 20 mg/kg Se and wool loss was observed during lactation in two ewes receiving 20 mg/kg Se. Livestock suffering from alkali disease were reported to have hair Se concentrations of up to 45 mg/kg and whole blood Se of 4.1 mg/L, while hooves, liver, and kidney of affected animals contained 10 mg/kg Se or more (NAS, 1983). At no time during our study did wool Se reach even 10 mg/kg and whole blood Se remained less than 50% of the aforementioned 4.1 mg/L concentration. Also, hoof Se remained under 8 mg/kg for all treatments during the course of our study. Liver and kidney Se concentrations from our study were higher than the 10 mg/kg previously reported. The elevated concentrations of Se in the liver and kidney of ewes consuming 16 and 20 mg/kg, and the observation of some clinical signs of Se toxicosis and limited pathological abnormalities in ewes consuming these Se levels may indicate that some ewes were beginning to suffer from Se toxicosis. However, definitive evidence was not observed. Therefore, it is necessary that either dietary Se concentration or duration of experiment be increased in order to induce a definitive Se toxicosis using inorganic Se.

Implications

The maximum tolerable level of selenium as sodium selenite for ruminants is higher than 2 mg/kg. Feeding up to 12 ppm selenite selenium to ewes under stresses of production (i.e., gestation and lactation) for 72 wk did not produce any clinical or

pathologic signs of selenium toxicosis. Ewes fed 16 and 20 mg/kg produced some signs of selenium toxicosis; however, general metabolic disorders could not be ruled out and no deaths of ewes consuming these levels of selenium were attributed to selenium toxicosis. Further studies of this nature should further prove that the current suggested tolerable level of Se is underestimated.

Summary

The objectives of this 72-wk study were to evaluate and compare the effects of six dietary levels of inorganic Se on serum, whole blood, wool, and tissue Se concentrations of mature ewes during lamb production and determine maximum tolerable level of Se during lamb production. Forty-one range-type ewes were used in a completely randomized design with six dietary treatments. Sodium selenite was added to a corn-soybean meal basal diet to provide 0.2 (control), 4, 8, 12, 16, and 20 mg/kg dietary Se to ewes during lamb production. Serum Se and ewe BW were measured at 4-wk intervals, whole blood Se, and wool Se were measured every 12 wk, and samples of brain, diaphragm, heart, hoof, kidney, liver, and psoas major muscle were collected at the termination of the experiment. Dietary Se did not affect ewe BW during the study ($P = 0.69$). Serum Se increased linearly as dietary Se level increased ($P < 0.001$) and responded cubically ($P = 0.02$) over time. Selenium in whole blood increased linearly ($P < 0.001$) with increased dietary Se and cubically ($P < 0.01$) over time. Wool Se increased linearly ($P < 0.001$) as dietary Se increased and response over time was quadratic ($P < 0.001$). Brain, diaphragm, heart, and psoas major muscle Se increased linearly as Se in the diet increased, liver Se responded quadratically, and hoof and kidney Se responded cubically to treatment ($P < 0.05$). In general, serum, whole blood, and tissue Se

concentrations from ewes receiving 12, 16, or 20 mg/kg dietary Se were higher ($P < 0.05$) than from controls and ewes receiving less dietary Se. Though serum, whole blood, and wool Se concentrations were elevated in ewes receiving increased dietary Se, at no time did serum, whole blood, or wool Se concentrations reach levels previously reported as toxic and a pattern of clinical signs of Se toxicosis was not observed. Microscopic evaluation of liver, kidney, diaphragm, heart, and psoas major muscle did not reveal evidence of Se toxicosis in ewes on any dietary Se level. Ewes under our experimental conditions and during the stresses of production were able to tolerate up to 20 mg/kg dietary Se as sodium selenite for 72 wk. These findings suggest that the maximum tolerable level of inorganic Se for sheep to be much higher than 2 mg/kg as was suggested previously. Experiments which are longer in duration and utilize higher dietary Se concentrations may be used to clearly define the maximum tolerable level.

Table 3-1. Diet composition (as-fed) for selenite-Se supplemented ewes[a]

Ingredient	% as-fed
Ground yellow corn	53.75
Cottonseed hulls	22.00
Soybean meal (47.5% CP)	16.00
Alfalfa meal (14% CP)	3.00
Soybean oil	3.00
Trace mineral mix[b]	1.00
Ground limestone	1.25
Vitamins A & D	—[c]

[a]Selenium levels in diet (as analyzed): 0.29, 3.77, 7.54, 11.01, 15.48, and 19.05 ppm for Se levels 0.2, 4, 8, 12, 16, and 20 ppm, respectively.
[b]Trace mineral mixture supplied between 96.5% and 98.5% NaCl, and provided per kg of diet: 1.0 mg Co (as carbonate), 5.0 mg Cu (as oxide), 0.7 mg I (as iodate), 35 mg Fe (as oxide), 25 mg Mn (as oxide), and 35 mg Zn (as oxide).
[c]Provided per kg of diet: 5,000 IU of Vitamin A and 500 IU of Vitamin D_3.

Table 3-2. Lamb production of ewes receiving different concentrations of dietary Se

	Year 1[a]		Year 2[b]	
Dietary Se, ppm	Ewes lambed	Lambs born[c]	Ewes lambed	Lambs born[d]
0.2	5	9	4	5
4	7	11	7	10
8	5	6	5	7
12	5	8	6	11
16	4	5	0	0
20	7	14	2	3
Total	33	53	24	36

[a]Ewes began receiving experimental diets at 57 d average gestation in yr 1.
[b]Ewes were fed experimental diets continuously during breeding and gestation yr 2.
[c]Lamb crop as lambs born (53) per ewe exposed (41) was 129% in yr 1.
[d]Lamb crop as lambs born (36) per ewe exposed (33) was 109% in yr 2.

Table 3-3. Effect of dietary inorganic Se level on serum Se concentration of mature ewes at various stages of lamb production[a]

	Dietary Se, mg/kg					
	0.2	4	8	12	16	20
Stage of Production	Serum Se, μg/L					
Late Gestation, yr 1[b]	$149^{g} \pm 67$	$242^{gh} \pm 67$	$354^{hi} \pm 79$	$414^{hi} \pm 79$	$463^{i} \pm 79$	$707^{j} \pm 81$
Lactation, yr 1[c]	$151^{g} \pm 56$	$272^{g} \pm 54$	$486^{h} \pm 63$	$623^{hi} \pm 63$	$718^{ij} \pm 63$	$811^{j} \pm 66$
Dry, rebreeding[d]	$162^{g} \pm 110$	$298^{gh} \pm 95$	$458^{hi} \pm 100$	$604^{i} \pm 99$	$1205^{j} \pm 106$	$1084^{j} \pm 113$
Late Gestation, yr 2[e]	$140^{g} \pm 137$	$313^{gh} \pm 124$	$446^{gh} \pm 142$	$596^{hi} \pm 142$	$986^{ij} \pm 158$	$1072^{j} \pm 149$
Lactation, yr 2[f]	$127^{g} \pm 114$	$325^{gh} \pm 103$	$536^{hi} \pm 114$	$718^{i} \pm 114$	$769^{i} \pm 136$	$1355^{j} \pm 121$

[a]Data represent least squares means ± SE.
[b]Late gestation, yr 1, defined as 56 d prepartum and includes serum Se concentrations for wk 4, 8, and 12.
[c]Lactation, yr 1, defined as 84 d postpartum and includes serum Se concentrations for wk 12, 16, 20, and 24.
[d]Dry, rebreeding period, 168 d, includes serum Se concentrations for wk 28, 32, 36, 40, 44, and 48.
[e]Late gestation, yr 2, defined as 56 d prepartum and includes serum Se concentrations for wk 52, 56, and 60.
[f]Lactation, yr 2, defined as 84 d postpartum and includes serum Se concentrations for wk 60, 64, 68, and 72.
[g,h,i,j]Means within rows lacking a common superscript differ ($P < 0.05$).

Table 3-4. Effect of dietary inorganic Se level on whole blood Se concentration of mature ewes[a]

	Dietary Se, mg/kg					
Week of	0.2	4	8	12	16	20
experiment	Whole blood Se, μg/L					
12[b]	$386^{h} \pm 168$	$839^{i} \pm 151$	$902^{ij} \pm 151$	$1241^{jk} \pm 139$	$1053^{ij} \pm 130$	$1558^{k} \pm 154$
24[c]	$420^{h} \pm 140$	$601^{hi} \pm 130$	$852^{ij} \pm 130$	$1047^{jk} \pm 130$	$1312^{k} \pm 130$	$1822^{l} \pm 154$
36[d]	$438^{h} \pm 152$	$635^{h} \pm 149$	$1079^{ij} \pm 138$	$1314^{ij} \pm 138$	$1668^{j} \pm 139$	$1373^{ij} \pm 154$
48[e]	$378^{h} \pm 154$	$661^{h} \pm 130$	$1117^{ij} \pm 139$	$1145^{j} \pm 130$	$1616^{j} \pm 151$	$1951^{j} \pm 154$
60[f]	$497^{h} \pm 154$	$802^{hi} \pm 138$	$1070^{ij} \pm 151$	$1131^{j} \pm 150$	$1892^{j} \pm 167$	$1796^{j} \pm 154$
72[g]	$410^{h} \pm 154$	$618^{hi} \pm 139$	$721^{hi} \pm 140$	$916^{ij} \pm 140$	$841^{hi} \pm 194$	$1855^{j} \pm 154$

[a]Data represent least squares means ± SE.
[b]n = 4, 5, 5, 6, 7, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[c]n = 6, 7, 7, 7, 7, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[d]n = 5, 5, 6, 6, 6, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[e]n = 5, 7, 6, 7, 5, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[f]n = 5, 6, 5, 5, 4, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[g]n = 5, 6, 6, 6, 3, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[h,i,j,k,l]Means within rows lacking a common superscript differ ($P < 0.05$).

Table 3-5. Effect of dietary inorganic Se level on wool Se concentration of mature ewes[a]

Week of experiment	Dietary Se, mg/kg					
	0.2	4	8	12	16	20
	Wool Se, mg/kg (DM basis)					
12[b]	$0.50^{h} \pm 0.58$	$0.71^{h} \pm 0.54$	$1.36^{hi} \pm 0.54$	$1.67^{h} \pm 0.54$	$2.00^{h} \pm 0.54$	$2.23^{i} \pm 0.64$
24[c]	$0.62^{h} \pm 0.62$	$1.43^{hi} \pm 0.54$	$2.76^{ij} \pm 0.54$	$2.79^{ij} \pm 0.54$	$3.58^{jk} \pm 0.54$	$4.64^{k} \pm 0.64$
36[d]	$1.58^{h} \pm 0.63$	$3.72^{i} \pm 0.54$	$4.86^{ij} \pm 0.54$	$3.96^{i} \pm 0.54$	$5.57^{j} \pm 0.57$	$5.27^{ij} \pm 0.64$
48[e]	$1.18^{h} \pm 0.64$	$4.86^{i} \pm 0.54$	$4.64^{i} \pm 0.54$	$4.82^{i} \pm 0.54$	$5.47^{i} \pm 0.57$	$5.53^{i} \pm 0.64$
60[f]	$1.25^{h} \pm 0.64$	$4.06^{i} \pm 0.54$	$6.09^{ij} \pm 0.57$	$5.50^{ij} \pm 0.57$	$5.63^{ij} \pm 0.68$	$5.17^{ij} \pm 0.64$
72[g]	$0.96^{h} \pm 0.64$	$3.42^{i} \pm 0.57$	$3.67^{ij} \pm 0.57$	$5.12^{j} \pm 0.58$	$5.11^{ij} \pm 0.80$	$7.69^{k} \pm 0.64$

[a]Data represent least squares means ± SE.
[b]$n = 6, 7, 7, 7, 7$, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[c]$n = 5, 7, 7, 7, 7$, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[d]$n = 5, 7, 7, 7, 6$, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[e]$n = 5, 7, 6, 7, 6$, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[f]$n = 5, 7, 6, 6, 4$, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[g]$n = 5, 6, 6, 6, 3$, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[h,i,j,k]Means within rows lacking a common superscript differ ($P < 0.05$).

Table 3-6. Amount of albumin and tissue enzyme activities present in serum of Se supplemented ewes[a,b,c]

Item	Normal concentration	Dietary Se, mg/kg					
		0.2	4	8	12	16	20
Albumin	2.4-4.0 g/dL	2.9	2.5	1.940	2.2	2.3	2.6
Alk Phos	68-387 IU/L	91.0	60.2	65.3	95.5	36.3	89.0
AST	60-280 IU/L	53.8	131.7	32.8	52.3	64.0	24.6
ALT	11-40 IU/L	15.4	34.7	2.7	13.3	17.7	24.0
GGT	15-60 IU/L	67.6	63.2	39.8	55.0	63.7	59.6
CK	0-584 IU/L	67.4	108.2	36.3	58.7	47.7	55.0

[a]Serum sample collected at wk 72.
[b]Albumin and tissue enzyme activities presented in same units as normal concentration ranges.
[c]GGT and CK ranges were established by University of Florida Veterinary Teaching Hospital.

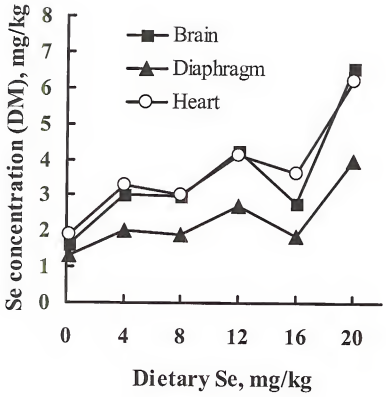

Figure 3-1. Effect of dietary inorganic Se level on Se concentrations in brain, diaphragm, and heart of ewes; SE = 0.6 to 0.9, 0.3 to 0.4, and 0.4 to 0.6 for brain, diaphragm, and heart, respectively.

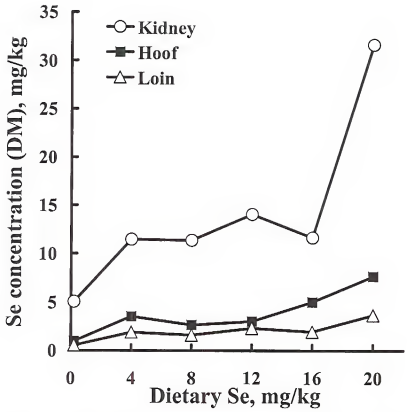

Figure 3-2. Effect of dietary inorganic Se level on Se concentrations in kidney, hoof, and loin (psoas major muscle) of ewes; SE = 3.0 to 3.3, 0.8 to 1.1, and 0.3 to 0.5 for kidney, hoof, and loin, respectively.

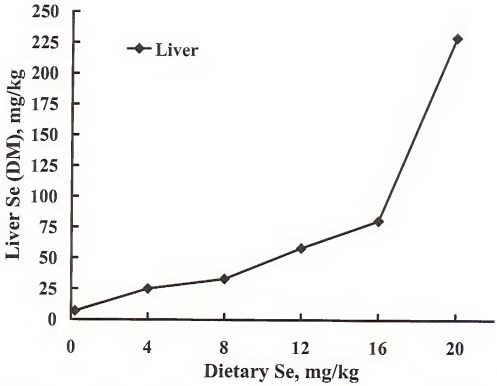

Figure 3-3. Effect of dietary inorganic Se level on liver Se concentration in ewes; SE = 27.5, 25.4, 24.5, 24.5, 34.6, and 26.9 for 0.2, 4, 8, 12, 16, and 20 mg/kg dietary Se, respectively.

CHAPTER 4
EFFECTS OF SELENIUM LEVELS IN EWE DIETS ON SELENIUM IN MILK AND PLASMA AND TISSUE SELENIUM CONCENTRATIONS OF LAMBS

Introduction

Selenium has long been implicated as a toxic element to livestock (Oldfield, 2002). Animals grazing seleniferous plants in certain regions of the world are subject to Se toxicosis and conditions such as alkali disease and "blind staggers" (McDowell, 2003). The estimated maximum tolerable level of Se for ruminant livestock is 2 mg/kg (NRC, 1980). However, recent research (Cristaldi et al., in press) has shown that sheep may consume up to 10 mg/kg Se as sodium selenite in the total diet for one yr, without showing signs of selenium toxicosis. Although it was concluded that these wethers were not suffering from Se toxicity, they did have increased serum, whole blood, and tissue Se concentrations. Like blood and tissue, milk Se is affected by dietary Se level (Conrad and Moxon, 1979; Givens et al., 2004) and Se readily crosses the placenta to the fetus (Van Saun et al., 1989). Furthermore, positive correlations exist between blood Se of cows and blood Se of their calves (Kincaid and Hodgson, 1989; Enjalbert et al., 1999; Pehrson et al., 1999). In sheep, Cuesta et al. (1995) showed increased colostrum Se from ewes receiving supplemental Se, and that milk Se was higher after one mo of supplementation. Thus, it seems that neonates from dams consuming high dietary levels of Se would have increased blood Se at birth, and subsequently would be exposed to high Se intake from increased Se in milk.

Acute Se toxicosis has been evaluated by injecting ewe lambs with sodium selenite (Blodgett and Bevill, 1987), and Abdennebi et al. (1998) evaluated the toxic effects of dosing lambs with extracts of milk vetch (*Astragalus lusitanicus*). In both studies, weaned lambs were utilized. Newborn and pre-weaned lambs differ from older sheep in ruminal and digestive function (Church, 1979; Goursand and Nowak, 1999), and may respond differently than older animals to increased Se intake. We hypothesized that when Se in gestating ewe diets is increased, colostrum Se, milk Se and plasma Se of their lambs will increase. The objective of this experiment was to follow ewes through two lamb crops and evaluate and compare the effects of six levels of dietary Se on ewes' milk and the Se status of their lambs prior to weaning.

Materials and Methods

All animal procedures were conducted within the guidelines of and approved by the University of Florida Institutional Animal Care and Use Committee. This 504-d experiment utilizing ewes and two lamb crops was conducted from December 18, 2001 to May 5, 2003 at the University of Florida Sheep Nutrition Unit located in southwestern Alachua County, FL. Thirty-three, four year old, Rambouillet ewes that originated from a single flock in Texas and were previously confirmed pregnant (average 57 d gestation) were weighed (57.4 ± 5.7 kg) and received 2-ml ivermectin (Ivomec; Merial Ltd. Iselin, NJ). Ewes were randomly assigned to one of six dietary treatments for a 504 d (two lambing seasons) study. Six dietary treatments were 0.2, 4, 8, 12, 16, or 20 mg/kg Se as sodium selenite (as-fed basis) added to a corn-soybean meal basal diet (Table 4-1). The basal diet was formulated to meet animal requirements for protein, energy as TDN, vitamins, and minerals for this class of sheep (NRC, 1985). Animal numbers per treatment were 5, 7, 5, 5, 4, and 7 for 0.2 (control), 4, 8, 12, 16, and 20 mg/kg added Se,

respectively. Ewes were housed by treatment group in covered wooden pens (53.5 m^2) with earth floors and ad libitum water. Diets were fed at 909 g/ewe/d from d 0 until lambing began (d 81), increased to 1000g/ewe/d during lambing (d 81 to 101), and again increased to 1135 g/ewe/d during lactation (d 101 to 171). Diets were sampled every 28 d, ground (1 mm), and frozen at 0°C until analysis.

In the first year, 52 lambs were born over 20 d from March 9, 2002 to March 28, 2002. Prior to lambing, ewes were fitted with a device to cover the udder and prevent lambs from nursing until a blood sample could be obtained. The udder cover was crafted from nylon pantyhose (Leggs Products, Winston-Salem, NC) and polyester elastic (2.54 cm wide) and held in place with safety pins (Figure 4-1). A blood sample for plasma analysis was collected from lambs immediately after birth via jugular venipuncture into 10-ml heparinized tubes (Vacutainer; Becton-Dickinson, Franklin Lakes, NJ). The udder cover was then removed from the ewe and five ml of pre-suckled colostrum was collected into a 15-ml plastic centrifuge tube (Fisher; Fisher Scientific, Pittsburgh, PA). Additional blood samples were collected from lambs and milk samples from ewes at 3, 28, and 56 d postpartum. Blood samples were centrifuged at 700 × *g* and the plasma then frozen at 0°C. Ewe milk samples were also stored frozen at 0°C for later analysis. Lambs were weaned at 70 d of age and ewes then received 909 g/ewe/d of their respective diets until next lambing. At 70 d, ram lambs were surgically castrated and the testes were frozen at 0°C until analysis.

On August 15, 2002, ewes were pen exposed to rams for 35 d. In the second year, 36 lambs were born over 34 d from January 17, 2003 to February 20, 2003. All sampling intervals, procedures, feeding levels, and materials used were duplicated from the first

year. Plasma, milk, testes, and feed samples from both years were analyzed for Se concentration using a fluorometric method described by Whetter and Ullrey (1978). To help ensure reliability of the analytical method, a certified standard (National Bureau of Standards Bovine Liver SRM-1577a; U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) was frequently analyzed.

Effects of treatment on lamb testicular and colostrum Se were analyzed using PROC MIXED in SAS (SAS for Windows 8e; SAS Inst. Inc., Cary, NC) in a completely randomized design. Contrast statements were used to compare means as described by Littell et al. (1998; 2000). PROC MIXED of SAS was also used to analyze effects of treatment, d, and the interaction of treatment × d on milk Se and plasma Se as repeated measures with a spatial power covariance structure with respect to d and a subplot of animal nested within treatment. Contrast statements were written to determine differences in means for different sampling d. PROC CORR was used to determine correlations of ewe milk Se to lamb plasma Se.

Results

In year one, 11 of 52 lambs were removed from the study before 56 d of age. Five lambs were born to ewes which produced little or no milk, one ewe had extremely enlarged or "bottle" teats and her two lambs were unable to suckle, two lambs died of weakness/dehydration, and two died of "joint ill." There were no apparent signs of selenium toxicosis in any lambs regardless of dietary Se level of their dams.

In year one, colostrum Se was affected by Se concentration of the ewes' diet ($P = 0.008$) and increased linearly ($P < 0.001$) as dietary Se increased (Table 4-2). Ewes receiving 16 or 20 mg/kg dietary Se produced higher ($P < 0.05$) colostrum Se than did controls. Ewes receiving 20 mg/kg dietary Se also produced higher ($P < 0.05$) colostrum

Se than did those ewes receiving 4 mg/kg dietary Se and tended ($P \leq 0.12$) to produce higher colostrum Se than ewes receiving 8 and 12 mg/kg dietary Se. Likewise, colostrum Se from ewes receiving 16 mg/kg tended to be higher ($P = 0.052$) than colostrum Se from ewes receiving 4 mg/kg dietary Se. Colostrum Se from ewes receiving 8, 12, or 16 mg/kg dietary Se was similar ($P \geq 0.20$).

In year two, 12 of 36 lambs were removed from the study before d 56. Seven lambs were removed due to their dams having either no milk or enlarged teats that were unable to be suckled, four lambs were lost to predation, and one lamb was removed due to physical injury. No lambs were lost or removed from the study due to dietary Se in the diet of their dam. As in year one, colostrum Se was affected by dietary Se ($P < 0.05$) and increased linearly ($P < 0.01$) as dietary Se increased (Table 4-2). Ewes receiving 8, 12, or 20 mg/kg dietary Se produced colostrum with similar ($P > 0.19$) Se concentrations, which were higher ($P < 0.05$) than colostrum Se from controls. Ewes consuming 8 mg/kg dietary Se produced colostrum Se higher ($P < 0.05$) than those ewes consuming 4 mg/kg dietary Se. Likewise, ewes consuming 20 mg/kg dietary Se tended to produce colostrum Se higher ($P = 0.10$) than ewes consuming 4 mg/kg dietary Se. No ewes receiving 16 mg/kg dietary Se lambed in year two and are not represented in these comparisons.

Ewe milk Se collected at 3, 28, and 56 d postpartum increased linearly ($P < 0.001$) as dietary Se increased in year one (Table 4-3). Day of sampling also had an effect ($P = 0.002$), but there was no treatment × d interaction. At d 3 postpartum, ewes receiving 4 and 8 mg/kg dietary Se produced similar ($P = 0.60$) milk Se and milk Se from ewes receiving 8 mg/kg dietary Se tended ($P = 0.06$) to be higher than from controls.

Ewes consuming 12, 16, and, 20 mg/kg Se produced milk Se higher ($P < 0.05$) than controls. Milk Se from ewes consuming 16 mg/kg Se tended to be higher ($P = 0.09$) than that from ewes consuming 20 mg/kg Se and was higher ($P < 0.05$) than milk Se from all other treatments. At d 28 postpartum, ewes consuming 20 mg/kg dietary Se produced milk Se concentrations higher ($P < 0.05$) than did controls or ewes consuming 4 mg/kg dietary Se. Likewise, milk Se from ewes consuming 20 mg/kg dietary Se tended to be higher ($P < 0.075$) than milk Se from ewes receiving 8 or 12 mg/kg Se. Milk Se from all other treatment groups was similar ($P > 0.20$). At the final milk collection in year one (d 56), milk Se concentrations from controls and ewes consuming 4, 8, and, 12 mg/kg Se were similar ($P > 0.17$). Ewes consuming 16 and 20 mg/kg Se produced similar milk Se ($P = 0.43$), which was higher ($P < 0.05$) than milk Se from all other treatments. A linear increase ($P < 0.01$) in milk Se as dietary Se increased was observed at each sampling d as well as over all sampling d. Milk Se concentrations in year one remained below 1000 μg/L from d 3 to d 56.

In year two, dietary Se concentration had an effect on milk Se ($P < 0.05$), as did the interaction of dietary Se concentration × sampling d ($P <0.05$). Milk Se concentrations from ewes consuming 8, 12, and 20 mg/kg Se were similar ($P \geq 0.38$) to each other and higher ($P < 0.05$) than controls at d 3 (Table 4-4). Ewes consuming 12 mg/kg Se had higher ($P < 0.05$) milk Se than did those consuming 4 mg/kg Se. Ewes consuming 20 mg/kg Se produced milk Se that tended ($P = 0.09$) to be higher than milk Se from ewes consuming 4 mg/kg Se. There were no differences ($P > 0.05$) in milk Se among treatment groups at d 28. However, ewes consuming either 12 or 20 mg/kg Se had milk Se which tended to be higher ($P \leq 0.12$) than control. At d 56, milk Se

concentrations from ewes consuming 20 mg/kg Se were higher than from all other treatments ($P < 0.05$). Ewes consuming 12 mg/kg Se had milk Se which was higher ($P < 0.05$) than controls and tended to be higher than from ewes receiving 4 mg/kg Se ($P = 0.07$). Milk Se concentrations, at d 56, from all other treatment groups were similar ($P > 0.18$). Milk Se concentrations increased linearly ($P < 0.001$) as dietary Se increased over all sampling d.

Lamb plasma Se was affected by dietary Se concentration of their dams ($P < 0.001$) and increased linearly as dietary Se of dams increased ($P < 0.001$) in year one (Table 4-5). Likewise, d of sampling affected lamb plasma Se concentration ($P < 0.01$). On d 3 to 56, lamb plasma Se was positively correlated to ewe milk Se ($r = 0.29$; $P < 0.001$). At birth, lambs suckling ewes consuming 20 mg/kg Se had higher plasma Se than controls ($P < 0.05$) and lambs suckling ewes consuming 16 mg/kg Se tended to have higher plasma Se than controls ($P = 0.15$). All other lambs had similar plasma Se ($P > 0.25$). At 3 d of age, lambs from ewes consuming 20 mg/kg Se had higher plasma Se ($P < 0.01$) than all other treatment groups. Plasma Se concentrations from control lambs were lower ($P < 0.05$) than plasma Se from lambs suckling ewes consuming 8, 12, or 16 mg/kg Se. At 28 d of age, lambs suckling ewes receiving 12, 16, or 20 mg/kg Se had higher plasma Se than did controls ($P < 0.01$). Likewise, lambs suckling ewes receiving 4 or 8 mg/kg Se tended to have higher plasma Se than controls ($P \leq 0.14$). Plasma Se from lambs suckling ewes receiving 20 mg/kg Se was higher than from lambs suckling dams that received 4, 8, or 12 mg/kg Se ($P < 0.05$) and tended to be higher than from lambs suckling dams that received 16 mg/kg Se ($P = 0.09$). At 56 d, plasma Se from lambs suckling ewes receiving 4, 12, 16, or 20 mg/kg Se was higher than controls ($P <$

0.05) and lambs suckling ewes that received 8 mg/kg Se tended to have higher plasma Se than controls ($P = 0.067$). Plasma Se from lambs suckling ewes receiving 16 or 20 mg/kg Se was higher than plasma Se from lambs suckling ewes receiving 4 mg/kg Se ($P < 0.05$) and tended to be higher than plasma Se from lambs suckling ewes receiving 8 mg/kg Se ($P \leq 0.08$).

Lamb plasma Se, in year two (Table 5-6), was affected by the concentration of Se in the diet of their dams ($P < 0.001$) and increased linearly as Se concentration in dams' diet increased ($P < 0.001$). Day of sampling and the interaction of dietary Se concentration × d of sampling also affected lamb plasma Se ($P < 0.01$). At birth, lambs from ewes receiving 12 mg/kg Se had higher plasma Se than did controls ($P < 0.05$). Likewise, lambs from ewes receiving 20 mg/kg Se had higher ($P < 0.05$) plasma Se than did controls and lambs from dams receiving 4 or 8 mg/kg Se. From 3 to 56 d of age, lamb plasma Se was positively correlated to ewe milk Se ($r = 0.44$; $P < 0.001$). At 3 d of age, lambs from all treatment groups had higher plasma Se than did controls ($P < 0.05$) and lambs suckling ewes receiving 8, 12, or 20 mg/kg Se had higher plasma Se than did those suckling ewes receiving 4 mg/kg Se ($P < 0.05$). At d 28, lamb plasma Se from all treatment groups was higher than controls ($P < 0.05$). Lambs suckling ewes receiving 20 mg/kg Se had plasma Se higher than all other treatment groups ($P < 0.05$). Also, lambs suckling ewes receiving 12 mg/kg Se had plasma Se higher than lambs suckling ewes receiving 4 or 8 mg/kg Se ($P < 0.05$). At d 56, lambs suckling dams receiving 4, 8, or 12 mg/kg Se had higher plasma Se than did controls ($P < 0.05$) and lambs suckling dams receiving 20 mg/kg Se tended to have higher plasma Se than did controls ($P = 0.067$).

Plasma Se from lambs suckling ewes receiving 12 mg/kg Se was higher than ($P < 0.05$) than plasma Se from lambs suckling ewes receiving 4 and 8 mg/kg.

Selenium concentration in testis (dry basis) taken from ram lambs at 70 d of age (weaning) increased linearly ($P < 0.001$) as dams' dietary Se concentration increased (Figure 4-2) in year one. Testicular Se from lambs suckling ewes receiving 20 mg/kg Se was higher than testis Se from controls and lambs suckling ewes receiving 4 or 8 mg/kg Se ($P < 0.05$). Lambs suckling ewes receiving 16 mg/kg Se had testicular Se which was higher ($P < 0.05$) than testicular Se from controls and lambs suckling ewes receiving 4 mg/kg Se. Lambs suckling ewes receiving 12 mg/kg tended to have higher testicular Se than did controls or lambs suckling ewes receiving 4 mg/kg Se ($P \leq 0.11$). Likewise, lambs suckling ewes receiving 8 mg/kg tended to have higher testicular Se than did controls ($P = 0.14$). There was no effect of treatment on testicular Se in year two ($P = 0.70$). Testicular Se concentrations were 2.05, 3.16, 2.96, and 3.24 mg/kg for controls and lambs suckling ewes receiving 4, 8, and 12 mg/kg Se, respectively.

Discussion

Colostrum Se increased with dietary Se level in both years. Cuesta et al. (1995) reported higher colostrum Se from vitamin E + Se supplemented ewes versus their unsupplemented counterparts. These findings are further supported by Mahan (2000), who demonstrated that colostrum Se was increased by increasing Se in prepartum and postpartum sow diets. Colostrum Se from Se supplemented crossbred ewes was increased over unsupplemented controls (Norton and McCarthy, 1986), however, those researchers used injectable vitamin E + Se as the supplemental Se source rather than dietary Se. Overnes et al. (1985) also reported an effect on colostrum Se from ewes receiving Se fed via free-choice salt and mineral mixtures. In the present study, ewe

colostrum Se concentrations from controls in year one were lower at 257 μg/L than values in cow colostrum from Romania reported by Serdaru et al. (2004). However, in year two, after our ewes had been receiving their respective diets for approximately 13 mo, colostrum Se from controls had more than doubled to 705 μg/L. The increase in colostrum Se after a longer duration of Se supplementation is substantiated by Maus et al. (1980). Those authors reported that Se in cows' milk increased with time when fed at 0.2, 0.3, 0.4, and 0.7 mg/kg in a corn-brewers' grain dairy diet. As dietary Se was increased by increments of 4 mg/kg from 4 mg/kg up to 20 mg/kg, colostrum Se increased by 45.3, 8.8, 55.2, and 10.1%, respectively in year one. Colostrum Se was numerically higher in year two when Se was fed at 0.2, 4, 8, and 12 mg/kg. This furthers the idea that colostrum Se, when Se is supplemented at equivalent concentrations, may be increased as animals are supplemented for an extended period of time. The use of increased Se in gestating animals may prove beneficial to their offspring as it provides greater antioxidative protection through increased colostrum Se and thus provides greater phagocytic and microbicidal activity (Wuryastuti et al., 1993).

As with colostrum Se, subsequent milk Se also increased as dietary Se increased. Givens et al. (2004) reported increased milk Se as selenite Se increased from 0.38 to 1.14 mg/kg and that a strong positive correlation exists between milk Se and dietary Se ($r = 0.979$). Gardner and Hogue (1967) reported up to five-fold increases in milk Se when sodium selenite was added to ewe diets at 1 mg/kg. In the present study, a five-fold or greater increase was observed in milk Se as dietary Se was increased from control (0.2 mg/kg) to 8 mg/kg for d 3, from control (0.2 mg/kg) to 20 mg/kg for d 28, and from control (0.2 mg/kg) to 12 mg/kg for d 56 in year one. In year two, a four-fold or greater

increase in milk Se was observed as dietary Se increased from control to 8 mg/kg on each sampling d. Few increases of that magnitude were observed between groups receiving Se at high concentrations. This indicates that the proportion of Se transferred to milk decreases as dietary Se concentration increases. Waite et al. (1975) suggest that Se is subject to a bioreducing process as it is transferred from plasma to milk. These authors report that only 1.5% of dietary selenite Se appeared in milk. Givens et al. (2004) observed increases of more than four-fold in cows' milk Se when dietary Se was doubled and tripled using Se yeast. However, in our study, increases of such magnitude were not observed when dietary Se was doubled and tripled using sodium selenite. These observations would indicate that, though milk Se can be increased using an inorganic Se source, a greater proportion of organic Se is transferred to milk. This concept is supported by several studies using cattle (Knowles et al., 1999; Ortman and Pehrson, 1999; Pehrson et al., 1999).

One objective of this study was to quantitate the effects on lambs that were suckling ewes that received dietary Se above the maximum tolerable level of 2 mg/kg (NRC, 1980). No lambs were born with congenital deformities or abnormalities, nor did any lamb display signs of selenium toxicosis (e.g., wool loss, hoof malformation, anorexia) from birth to weaning. Selenium included in ewe diets has previously been shown to be transmitted to the lamb via the placenta and milk (Jacobsson et al., 1965). In our study, plasma Se in lambs increased as Se concentration in their dams' diet increased and was positively correlated to milk Se. Lambs from ewes receiving the control diet had plasma Se at birth that averaged 81 μg/L in year one and 85μg/L in year two. These values are only slightly above the normal range (50-80 μg/L) for neonate lambs (Aitken,

2001) and more than double the plasma Se concentration suggested by Bostedt and Schramel (1990) for normal growth and health in newborn calves. Lamb plasma, collected before nursing, increased in Se as Se concentration in the diet fed to ewes during gestation increased in both years. Ewes receiving 12 mg/kg dietary Se gave birth to lambs with up three-fold higher plasma Se than did controls. Likewise, ewes receiving 20 mg/kg dietary Se, which is ten fold higher than the established maximum tolerable level for Se, gave birth to lambs with only approximately four-fold higher plasma Se than did controls. These results indicate that Se does cross the placenta to the fetus. Koller et al. (1984) demonstrated maternal transfer of Se in beef cattle and Kim and Mahan (2001) reported elevated serum and tissue Se in neonate pigs when dietary Se levels of sows were increased. This does not concur with (Wright and Bell, 1964) who reported no increase in lamb plasma Se when their dams were fed increased Se and demonstrated a defined placental barrier for Se.

Plasma Se remained elevated in lambs which were suckling ewes receiving increased dietary Se and from d 3 to d 56 ranged from 196 to 648 μg/L in year one and 244 to 775 μg/L in year two. These plasma Se concentrations were much higher than the > 70 μg/L suggested as adequate by Zachara et al. (1993). However, at no time did any lamb have plasma Se near or above 1400 μg/L which has been suggested as the plasma level when signs of Se toxicosis appear in sheep (Glenn et al., 1964c) and swine (Kim and Mahan, 2001). Marrow (1968) reported that death occurred within 16 hours in 35% of nursing lambs which were dosed with 10 mg of sodium selenite orally in an attempt to prevent nutritional muscular dystrophy. Smyth et al. (1990) observed death as soon as six hours after an oral dose of 5 mg Se/kg BW. Contrarily, Lagace et al.(1964) dosed

lambs from two to 14 wk of age with 5 mg of sodium selenite via subcutaneous injection every two wk and did not induce Se toxicity. Lambs on our study did not receive nearly the amount of Se that others reported to be deadly, even from nursing dams supplemented with Se up to 20 mg/kg during gestation and lactation. However, our lambs were subjected to elevated milk Se concentrations. Based on data from Mellor and Murray (1986) and Wohlt et al. (1984) milk intake in lambs from birth to 56 d ranges from 866-1246 g/d. Given those intake estimates and the colostrum and milk Se concentrations from the present study, lambs consuming the colostrum or milk with the highest Se concentration at the highest intake would ingest 4.39 mg of Se/d. In newborn lambs (3 kg BW), that amount of Se would translate to 1.46 mg Se/kg BW and to 0.29 mg Se/kg BW in 8 wk old lambs (15 kg BW). These levels are considerably less than levels previously reported to cause death in young lambs.

Testes taken from ram lambs at 70 d were evaluated for Se concentration. Selenium is implicated in sperm quality and reproductive function of livestock (Hidiroglou, 1982; Marin-Guzman et al., 2000) and concentrations in testes are less than in liver and generally greater than in heart, spleen, and pancreas. As with plasma Se in the suckling lambs, testicular Se of lambs increased as Se increased in the ewe diets and ranged from 1.67 mg/kg in controls to 4.25 mg/kg in lambs whose dams received 20 mg/kg dietary Se. These Se concentrations lie between those concentrations found in the liver and heart of wethers consuming up to 10 mg/kg Se as sodium selenite for one year (Cristaldi et al., in press). Those wethers were reported to also have elevated concentrations of Se in serum, whole blood, wool, and other organs. However, they displayed no clinical signs of selenium toxicosis

Implications

Feeding Se to gestating and lactating ewes above the current maximum tolerable level (2 mg/kg) does increase the Se concentration in colostrum and subsequently produced milk. However, this practice does not increase milk Se concentrations to a level at which their nursing lambs suffer from Se toxicosis. Likewise, feeding increased Se to ewes does increase plasma and tissue Se in lambs but not to a concentration above those previously found in sheep determined not to be suffering from Se toxicity. Moreover, data from other species even suggests that feeding increased Se to gestating and lactating animals may produce colostrum of higher quality that may be beneficial to their offspring. Data from this and other recent research has now established that the maximum tolerable level of Se, as selenite, for sheep to be considerably higher than the previously suggested 2 mg/kg.

Summary

The objective of this 504-d experiment was to evaluate and compare the effects of six levels of dietary selenium (Se) on ewes' milk and the Se status of their lambs prior to weaning. Sodium selenite was added to a basal diet to provide 0.2 (control), 4, 8, 12, 16, and 20 mg/kg dietary Se for ewes during gestation and lactation over two lambings. Colostrum Se ranged from 257 to 3542 μg/L and increased linearly as dietary Se increased ($P < 0.001$) in both years. Ewe milk Se ranged from 75 to 2228 μg/L and also increased linearly as dietary Se increased ($P < 0.01$). In general, ewes receiving ≥ 12 mg/kg Se produced higher milk Se than controls. Blood samples were collected from lambs before nursing and at 3, 28, and 56 d of age to evaluate plasma Se concentrations. At birth, lamb plasma Se ranged from 74 to 775 μg/L and was affected ($P < 0.001$) by the Se concentration of the ewe diets, which indicates placental transfer of Se. Lambs from

ewes receiving dietary Se at 20 mg/kg had higher ($P < 0.05$) plasma Se than controls at birth and 3, 28, and 56 d of age in both years. Selenium concentration in lamb testes collected at 70 d of age was also affected by Se content of ewe diets. In year one, lambs whose dams received 16 or 20 mg/kg Se had higher ($P < 0.05$) testicular Se than controls, and no differences in testicular Se were observed in year two. No signs of Se toxicosis were observed in lambs regardless of dietary Se concentration of the ewes' diet. These results suggest that ewes consuming up to 20 mg/kg inorganic Se may give birth to normal lambs, and that the lambs may not suffer from Se toxicosis before weaning. Selenium as sodium selenite may be fed to ewes at concentrations greater than the current maximum tolerable levels (2 mg/kg) without adversely affecting their offspring.

Table 4-1. Diet composition (as-fed) for Se (selenite) supplemented ewes[a]

Ingredient	% as-fed
Ground yellow corn	53.75
Cottonseed hulls	22.00
Soybean meal (47.5% CP)	16.00
Alfalfa meal (14% CP)	3.00
Soybean oil	3.00
Trace mineral mix[b]	1.00
Ground limestone	1.25
Vitamins A & D	—[c]

[a]Selenium levels in diet (as analyzed): 0.29, 3.77, 7.54, 11.01, 15.48, and 19.05 ppm for treatments 0.2, 4, 8, 12, 16, and 20 ppm, respectively.
[b]Trace mineral mixture supplied between 96.5% and 98.5% NaCl, and provided per kg of diet: 1.0 mg Co (as carbonate), 5.0 mg Cu (as oxide), 0.7 mg I (as iodate), 35 mg Fe (as oxide), 25 mg Mn (as oxide), and 35 mg Zn (as oxide).
[c]Provided per kg of diet: 5,000 IU of Vitamin A and 500 IU of Vitamin D_3.

Table 4-2. Colostrum selenium concentrations (μg/L) of ewes receiving different levels of selenium supplementation as sodium selenite[a]

	Year of experiment	
Added Se, mg/kg	1[1]	2[2]
0.2	$257^{b} \pm 624$	$705^{b} \pm 517$
4	$1300^{bc} \pm 543$	$1452^{bd} \pm 421$
8	$1889^{bcd} \pm 767$	$3256^{c} \pm 480$
12	$2072^{bcd} \pm 704$	$2373^{cd} \pm 462$
16	$3216^{cd} \pm 767$	—
20	$3542^{d} \pm 537$	$2925^{cd} \pm 741$

[a]Data represent least squares ± standard errors.
[b,c,d]Means within columns lacking a common superscript differ ($P < 0.05$)
[1]n = 4, 5, 3, 3, 3, and 6 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[2]n = 4, 6, 5, 5, and 2 for Se levels 0.2, 4, 8, 12, and 20 mg/kg, respectively.

Table 4-3. Milk Se concentrations (μg/L) from ewes receiving different levels of dietary Se as sodium selenite in year one[a]

	Days postpartum		
Added Se, mg/kg	3[1]	28[2]	56[3]
0.2	$75^{b} \pm 117$	$66^{b} \pm 91$	$32^{b} \pm 91$
4	$312^{bc} \pm 117$	$121^{b} \pm 91$	$165^{b} \pm 83$
8	$400^{bc} \pm 117$	$163^{bc} \pm 117$	$160^{b} \pm 117$
12	$490^{c} \pm 144$	$189^{bc} \pm 117$	$241^{b} \pm 117$
16	$920^{d} \pm 117$	$253^{bc} \pm 144$	$653^{c} \pm 117$
20	$628^{cd} \pm 117$	$466^{c} \pm 91$	$538^{c} \pm 83$

[a]Data represent least squares means ± SE.
[b,c,d]Means within columns lacking a common superscript are different ($P < 0.05$).
[1]n = 3, 3, 3, 2, 3, and 3 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[2]n = 5, 5, 3, 3, 2, and 5 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.
[3]n = 5, 6, 3, 3, 3, and 6 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.

Table 4-4. Milk Se concentrations (μg/L) from ewes receiving different levels of dietary Se as sodium selenite in year two[a]

	Days postpartum		
Added Se, mg/kg	3[1]	28[2]	56[3]
0.2	$57^{b} \pm 238$	$81^{b} \pm 238$	$69^{b} \pm 238$
4	$574^{bc} \pm 194$	$646^{b} \pm 180$	$339^{bc} \pm 194$
8	$1442^{d} \pm 213$	$462^{b} \pm 213$	$493^{bc} \pm 213$
12	$895^{cd} \pm 238$	$689^{b} \pm 238$	$914^{c} \pm 238$
20	$933^{cd} \pm 337$	$923^{b} \pm 475$	$2228^{d} \pm 476$

[a]Data represent least squares means ± SE.
[b,c,d]Means within columns lacking a common superscript are different ($P < 0.05$).
[1]n = 4, 5, 5, 5, and 2 for Se levels 0.2, 4, 8, 12, and 20 mg/kg, respectively.
[2]n = 4, 6, 5, 4, and 1 for Se levels 0.2, 4, 8, 12, and 20 mg/kg, respectively.
[3]n = 4, 5, 5, 4, and 1 for Se levels 0.2, 4, 8, 12, and 20 mg/kg, respectively.

Table 4-5. Plasma Se concentrations (μg/L) of lambs suckling ewes receiving different levels of dietary Se as sodium selenite in year one[a]

	Age of lamb, d			
Se in ewe diet,mg/kg	0[1]	3[2]	28[3]	56[4]
0.2	$81^{b} \pm 66$	$111^{b} \pm 66$	$76^{b} \pm 52$	$92^{b} \pm 52$
4	$127^{bc} \pm 60$	$204^{bc} \pm 56$	$196^{b} \pm 49$	$246^{c} \pm 49$
8	$131^{bc} \pm 85$	$330^{c} \pm 73$	$209^{bc} \pm 73$	$258^{bcd} \pm 73$
12	$188^{bc} \pm 66$	$333^{c} \pm 85$	$374^{cd} \pm 66$	$354^{cd} \pm 66$
16	$238^{bc} \pm 85$	$387^{c} \pm 73$	$297^{c} \pm 73$	$431^{d} \pm 66$
20	$294^{c} \pm 47$	$648^{d} \pm 60$	$508^{d} \pm 44$	$419^{d} \pm 47$

[a]Data represent least squares means ± SE.

[b,c,d]Means within columns lacking a common superscript are different ($P < 0.05$).

[1]n = 5, 6, 3, 5, 3, and 6 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.

[2]n = 5, 7, 4, 3, 4, and 6 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.

[3]n = 8, 9, 4, 5, 5, and 11 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.

[4]n = 8, 9, 4, 5, 5, and 10 for Se levels 0.2, 4, 8, 12, 16, and 20 mg/kg, respectively.

Table 4-6. Plasma Se concentrations (μg/L) of lambs suckling ewes receiving different levels of dietary Se as sodium selenite in year two[a]

	Age of lamb, d			
Se in ewe diet,mg/kg	0[1]	3[2]	28[3]	56[4]
0.2	$85^{b} \pm 62$	$74^{b} \pm 55$	$81^{b} \pm 55$	$86^{b} \pm 55$
4	$182^{bc} \pm 41$	$325^{c} \pm 44$	$244^{c} \pm 47$	$287^{c} \pm 47$
8	$186^{bc} \pm 47$	$601^{d} \pm 47$	$314^{cd} \pm 55$	$263^{c} \pm 51$
12	$253^{cd} \pm 41$	$686^{d} \pm 51$	$553^{e} \pm 62$	$430^{d} \pm 55$
20	$353^{d} \pm 71$	$737^{d} \pm 71$	$775^{f} \pm 87$	$340^{bcd} \pm 124$

[a]Data represent least squares means ± SE.

[b,c,d,e,f]Means within columns lacking a common superscript are different ($P < 0.05$).

[1]n = 4, 9, 7, 9, and 3 for Se levels 0.2, 4, 8, 12, and 20 mg/kg, respectively.

[2]n = 5, 8, 7, 6, and 3 for Se levels 0.2, 4, 8, 12, and 20 mg/kg, respectively.

[3]n = 5, 7, 5, 4, and 2 for Se levels 0.2, 4, 8, 12, and 20 mg/kg, respectively.

[4]n = 5, 7, 6, 5, and 1 for Se levels 0.2, 4, 8, 12, and 20 mg/kg, respectively.

Figure 4-1. Rambouillet ewe in late gestation fitted with a device to cover the udder and prevent lambs from suckling until a blood sample was obtained. Device was made from nylon pantyhose and elastic straps, and held in place with safety pins. (Device courtesy of Dr. Donald J. Davis, Crossville, TN)

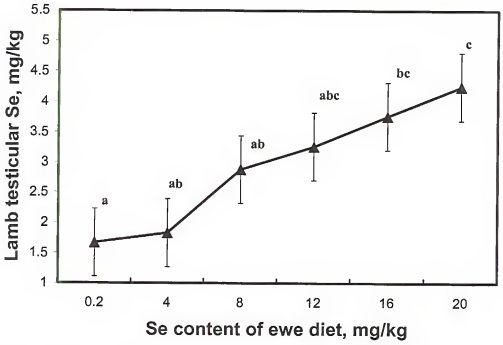

Figure 4-2. Effects of Se concentration of ewe diet on testicular Se concentration of their lambs in year one. Testicular selenium concentrations were 1.67, 1.83, 2.88, 3.26, 3.76, and 4.25 mg/kg (dry matter basis) for ram lambs suckling ewes receiving 0.2, 4, 8, 12, 16, and 20 mg/kg dietary Se, respectively. SE = 0.56.

CHAPTER 5
COMPARATIVE EFFECTS AND TOLERANCE OF VARIOUS DIETARY LEVELS OF SE AS SODIUM SELENITE OR SE YEAST ON BLOOD, WOOL, AND TISSUE SE CONCENTRATIONS OF WETHER SHEEP

Introduction

Selenium was first implicated as an essential nutrient for animals by Schwarz and Foltz (1957). Prior to that, Se was viewed primarily as a detriment to livestock which was documented by Franke (1934) and Moxon (1937). Selenium deficiency is far more prevalent worldwide than toxicity. However, Se toxicity is a greater concern to livestock producers and nutritionists, as toxicities are more difficult than deficiencies to control. Current estimates put the maximum tolerable level of Se at 2 mg/kg for the major livestock species (NRC, 1980) and no differentiation exists for tolerable levels between ruminants and monogastric animals. However, previous research suggests that inorganic Se (e.g., sodium selenite) may be reduced to insoluble selenide by microorganisms in the rumen, thus reducing overall absorption of Se by ruminant animals (Butler and Peterson, 1961; Hidiroglou et al., 1968). Wright and Bell (1966) reported that swine retained 77% and sheep retained 29% of an oral dose of inorganic Se. The NRC makes no distinction between inorganic and organic (e.g., Se yeast or seleno-methionine) forms of Se for current maximum tolerable levels, though the chemical form of dietary Se leads to markedly different physiological responses of livestock (Knowles et al., 1999; Pehrson et al., 1999; Gunter et al., 2003). Kim and Mahan (2001) reported more accumulation of Se in the plasma and tissues of swine fed high dietary levels of Se as Se yeast compared to the same Se levels as sodium selenite, and that Se toxicity occurred sooner and its clinical

signs were more severe when inorganic Se was used as the dietary source. In concluding that > 5 mg/kg dietary Se, regardless of source, did produce signs of Se toxicity in growing swine, those authors postulated that the greater tissue retention of organic Se may reduce the incidence of Se toxicity. Based on these findings and the increasing use of organic forms of Se for supplementation to livestock, an experiment was conducted to evaluate and compare effects of feeding Se as sodium selenite or Se yeast at high dietary levels on serum, whole blood, wool, and tissue Se concentrations of wether sheep.

Materials and Methods

All animal procedures were conducted within the guidelines of and approved by the University of Florida Institutional Animal Care and Use Committee. This experiment was conducted from June 4, 2002 to July 29, 2003 at the University of Florida Sheep Nutrition Unit located in southwestern Alachua County, FL. Twenty-eight, 2-yr-old, Rambouillet-crossbred wethers were weighed (62.3 ± 8.5 kg) and received 2-ml ivermectin dewormer s.c. (Ivomec; Merial Ltd. Iselin, NJ). Wethers were randomly assigned to one of eight dietary treatments for a 60-wk study. Dietary treatments were arranged as a 2 × 4 factorial with 0.2, 20, 30, and 40 mg/kg Se (as-fed) as four dietary levels and Se yeast and sodium selenite as two Se sources added to a corn-soybean meal-cottonseed hull basal diet (Table 5-1). Feed-grade yeast was used as a carrier for the sodium selenite in order to alleviate differences in the palatability and protein content of the diets. The basal diet was formulated to meet animal requirements for protein, energy as TDN, vitamins, and minerals for this class of sheep (NRC, 1985). Animal numbers per treatment were three for 0.2 (control) and 20 mg/kg Se, and four each for 30 and 40 mg/kg Se treatments for both Se sources. Wethers were housed by treatment group in covered wooden pens (53.5 m^2) with earth floors and ad libitum water.

Diets were fed at 909 g·wether^{-1}·d^{-1} throughout the experiment. Samples of each diet were taken every 28 d, ground (1 mm), and frozen at 0°C until analysis.

Wether BW was recorded on d 0 and for every eight wk thereafter, for the remainder of the study. A 10-mL blood sample for serum analysis was collected using an 18-gauge needle into a vacutainer tube with no additive (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) every 12 wk, via jugular venipuncture, allowed to stand for 20 min, centrifuged at 700 x g for 25 min, and serum stored frozen at 0°C until Se analysis. An additional 10 mL of blood was collected into a heparinized vacutainer tube (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). This additional 10-mL sample was also collected every 12 wk for the remainder of the experiment and stored frozen at 0°C as whole blood until analysis.

The wool around the jugular was shorn initially and regrowth was collected beginning at wk 12 and every 12 wk thereafter. The collected wool was washed with a commercial hair shampoo (Alberto VO5; Alberto-Culver Co., Melrose Park, IL), to remove oil and dirt, rinsed well with deionized water, dried, stored at room temperature, and later analyzed for Se.

At the termination of the experiment (wk 60), wethers were slaughtered by stunning and exsanguination, following USDA procedures at the University of Florida Meats Laboratory. Immediately prior to slaughter, a 10-mL sample of blood was collected using an 18-gauge needle into a vacutainer, centrifuged at 700 x g for 25 min, and serum frozen at 0°C for analysis of albumin and the following enzymes: alkaline phosphatase (Alk Phos), alanine transaminase (ALT), aspartate transaminase (AST), creatinine phosphokinase (CK), and gamma glutamyl transferase (GGT).

Samples of brain, diaphragm, heart, hoof tip, kidney, liver, and psoas major muscle were collected, and frozen (0°C) until analyzed for Se. Sections (1 cm^3) of liver, heart, kidney, diaphragm, and psoas major muscle from all animals were placed in 10% neutral-buffered formaldehyde for subsequent microscopic evaluation for evidence of Se toxicosis.

For histopathological evaluation, the tissue samples fixed in buffered formalin were embedded in paraffin and sectioned at 6 microns. All sections were stained with hematoxylin and eosin, and examined under a light microscope (10X, 20X, and 40X). Serum albumin, Alk Phos, ALT, AST, CK, GGT were evaluated on a Hitachi 911 analyzer with reagents from Sigma (Sigma Chemical Co., St. Louis, Mo.). These procedures were established by the Veterinary Medical Teaching Hospital at the University of Florida.

Samples of kidney, heart, and liver were evaluated for cell structure changes using transmission electron microscopy. Tissues were transferred to Trump's fixative, pH 7.2, for 2 h at room temperature (24° C). Samples were then rinsed in 0.1M sodium cacodylate buffer at room temperature for 1 h. After three 15-min rinses with deionized water, the tissues were placed in a 1% aqueous uranyl acetate solution for 45 min. Samples were then dehydrated through a graded ethanol-acetone series at room temperature. Tissue samples were then infiltrated with and embedded in Spurr's resin. Silver sections (0.06 um) were cut using an RMC MT 6000XL ultramicrotone (RMC Products; Boeckeler Instruments, Inc.,Tuscon, AZ) and mounted on formvar-coated copper mesh grids. Sections were stained with 5% acidic uranyl acetate and Reynold's lead citrate and examined in a Zeiss 100 microscope (Carl Zeiss, Inc, Thornwood, NY).

Serum, whole blood, wool, tissue, and feed samples were analyzed for Se concentration using a fluorometric method described by Whetter and Ullrey (1978). To help ensure reliability of the analytical method, a certified standard (National Bureau of Standards Bovine Liver SRM-1577a; U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) was frequently analyzed.

Brain, diaphragm, heart, hoof tip, kidney, liver, and psoas major muscle Se data were analyzed for effects of treatment using PROC GLM in SAS (SAS for Windows 8e; SAS Inst., Inc., Cary, NC) in a 2 × 4 factorial arrangement. Pre-planned orthogonal contrast statements were used to compare means as described by Littell et al. (1998; 2000). PROC MIXED of SAS was used to analyze effects of treatment, time, and the interaction of treatment × time on BW, serum Se, whole blood Se, and wool Se as repeated measures with a spatial power covariance structure with respect to d and a subplot of animal nested within treatment. Pre-planned orthogonal contrast statements were written to determine differences in means at different sampling intervals. Regression analysis was used to determine relationships between dietary Se and Se concentration in serum, whole blood, wool, and tissues.

Results and Discussion

Wether BW was affected by dietary Se level ($P < 0.05$), source of dietary Se ($P < 0.05$), time ($P < 0.05$), and average BW decreased linearly ($P < 0.10$) as dietary Se level increased (Table 5-2). Body weights of wethers receiving 30 or 40 mg/kg dietary Se as Se yeast decreased from wk 0 to wk 60, whereas wethers receiving all other dietary Se treatments gained weight from wk 0 to wk 60. Previous studies have reported no effect of Se fed above requirements on BW of feedlot cattle (Perry et al., 1975) and no effects on BW when included up to 10 mg/kg in the diets of wether sheep (Cristaldi et al., in

press). Likewise, Ullrey et al. (1977) reported that lamb BW were unaffected by dietary Se level in feeds containing differing proportions of organic and inorganic Se. However, Kim and Mahan (2001) reported a quadratic decrease in final BW of swine as dietary Se level was increased using sodium selenite or Se yeast. Those authors observed the most drastic decreases when selenite Se was added above 10 mg/kg and when Se yeast was added at 20 mg/kg. Our results differ from the findings with swine, as organic Se had a more dramatic deleterious affect on BW than did selenite Se. This could be explained by organic Se not being subject to reduction to selenides by rumen microorganisms as suggested by previous research (Whanger et al., 1968; van Ryssen et al., 1989; Whanger, 2002) and thus, being more available to cause toxic effects in ruminant livestock.

Seven of 28 wethers died during the course of our study and were subjected to gross necropsy by pathologists at the University of Florida Veterinary Teaching Hospital. All wethers were described in good physical condition at time of necropsy with adequate adipose tissue. Causes of death were determined to be spinal cord compression trauma, endoparasitism, pulmonary edema, and unknown. One wether from the 20 mg/kg organic Se group had mild hepatic lipidosis and one wether from the 30 mg/kg organic Se group exhibited signs of mild myocarditis. However, definitive evidence of death due to Se toxicosis was not found and the gross lesions seemed to be due to metabolic changes or were merely background findings.

Serum selenium values (105 ± 23 μg/L) were below the normal range (120 to 150 μg/L) for adult sheep (Aitken, 2001) and did not differ among treatment groups ($P = 0.36$) at the initiation of the experiment. Serum Se concentrations measured at wk 12, 24, 48, and 60 ranged from 110 to 3922 μg/L and increased linearly ($P < 0.05$) as dietary Se

level increased, while a quadratic response ($P < 0.05$) was observed at wk 36 (Table 5-3). Serum Se concentrations of wethers were affected by dietary Se level, Se source, and the interaction of dietary Se level × Se source interaction ($P < 0.05$). Likewise, over the entire trial serum Se increased quadratically ($P < 0.05$) as dietary Se level increased. Wethers receiving organic Se had higher ($P < 0.001$) serum Se than did selenite treated animals throughout the study. Kim and Mahan (2001) reported a linear increase in plasma Se of swine as dietary Se was increased as organic or inorganic Se. Likewise, those authors reported an effect of source of Se, with pigs supplemented with organic Se having higher plasma Se than their inorganically supplemented counterparts. Cristaldi et al. (2004) reported a linear increase in serum Se as dietary Se was increased, however those authors used a maximum level of 10 mg/kg dietary Se as selenite. The quadratic response observed in the present study suggests homeostatic regulation of Se in blood as dietary levels exceed 30 mg/kg. Serum Se in wethers fed up to 10 mg/kg selenite Se for 52 wk reached 870 μg/L (Cristaldi et al., in press). Wethers in the present experiment exceeded 870 μg/L when receiving either Se source at 20, 30, or 40 mg/kg and at wk 24 wethers receiving 30 mg/kg organic Se had more than four-fold higher serum Se than the maximum serum Se reported by Cristaldi et al. (2004). Our data show that at most collections organic Se produced serum Se of more than double the concentration produced by feeding selenite Se at the same level. Wethers receiving 20, 30 or 40 mg/kg organic Se had serum Se above 1500 μg/L throughout the experiment. Aitken (2001) reported serum Se of 1500 μg/L as a level at which signs of toxicity appear in horses. Likewise, Aitken (2001) reported that serum Se of 3700 μg/L was evident of Se toxicosis

in swine. At wk 24, wethers receiving 30 mg/kg organic Se had serum Se of 3922 μg/L. At no other time during our study did wether serum Se exceed 3700 μg/L.

Whole blood Se was measured in addition to serum Se because of the possibility of a more accurate Se measurement since use of whole blood eliminates the possibility of falsely high Se readings in serum due to hemolysis (Maas et al., 1992). Whole blood Se responded to Se supplementation in much the same fashion as did serum Se (Table 5-4). This response supports the high correlation between serum Se and whole blood Se previously described by Maas et al. (1992) and Cristaldi et al. (2004). Whole blood Se concentrations of wethers were affected by dietary Se level, Se source, and the interaction of dietary Se level × Se source interaction ($P < 0.05$) ranged from 392 to 6259 μg/L, and overall increased quadratically ($P < 0.01$) as Se concentration of the wether diets was increased. Whole blood Se concentrations measured at wk 12, 24, 48, and 60 increased linearly ($P < 0.10$) as dietary Se level increased. Whole blood Se in swine increased linearly as dietary Se as sodium selenite was increased from 0 to 20 mg/kg (Goehring et al., 1984b) and whole blood in sheep responded linearly to increased dietary Se (Cristaldi et al., in press). Likewise, those authors reported a strong correlation between Se concentrations of serum and whole blood. In the present study, whole blood Se responded in neither a linear nor quadratic manner at wk 36 ($P > 0.15$). It seems that organic Se was used in place of inorganic Se for the selenite control diet during that feeding period, which created a whole blood Se concentration of 1004 μg/L for the selenite control group at wk 36. On average, whole blood Se responded quadratically ($P < 0.05$) as dietary Se level was increased, again suggesting the influence of homeostatic regulation when dietary Se is increased above 30 mg/kg. Wethers receiving organic Se

had higher ($P < 0.001$) whole blood Se than did wethers receiving inorganic Se throughout the study. The maximum whole blood Se concentration observed during our study was 6259 μg/L. This concentration is well above the range of 2000 to 4000 μg/L for whole blood Se, where clinical signs of Se toxicosis should appear (Rosenfeld and Beath, 1945; 1946) and likewise is greater than a whole blood Se concentration of 4000μg/L, that Maag and Glenn (1967) described as the blood concentration above which steers became depressed and inactive. However, wethers on the present study, with the highest whole blood Se concentrations (> 6200 μg/L) did not exhibit signs of Se toxicosis (e.g. wool loss, anorexia, abnormal hoof growth). Glenn et al. (1964a) fed sodium selenate at high levels to range ewes that were similar in BW and breed type to the wethers on the present study. Those researchers did not induce death by Se toxicosis with daily oral doses less than 25 mg Se/ewe. Of the 17 deaths reported in their experiment, only one was induced with a daily dose of 25 mg Se/ewe. Eight deaths were induced with a daily dose 37.5 mg Se/ewe and eight deaths were induced with a daily dose 50 mg Se/ewe. Those reported deaths were not by acute Se toxicosis; rather the ewes received experimental Se doses for at least 80 d before death by Se toxicosis was induced. In the same experiment, Glenn et al. (1964a) further suggested an avg minimum toxic level of Se for adult sheep to be 0.825 mg/kg BW when fed for 100 d. Using this estimate, the minimum toxic level of Se for sheep of the size used in our study would be 51.4 mg/d. Selenium consumption of wethers receiving the highest dietary Se level (40 mg/kg) was 78% of the aforementioned minimum toxic level for sheep. Blodgett and Bevill (1987) reported the LD_{50} for sheep, using sodium selenite via i.m. injection, to be 0.7 mg Se/kg BW. Wethers of avg BW, on our study, receiving 1 kg of diet containing

40 mg/kg Se received 91.1% of that LD_{50} for sheep throughout the experiment. Furthermore that LD_{50} for sheep (Blodgett and Bevill, 1987) was established using injectable Se. Administration of Se parenterally disallows the reduction of selenite Se to insoluble selenide via ruminal microorganisms as described by (Whanger et al., 1968). This would suggest that the LD_{50} for Se in sheep could be considerably higher than previously thought.

Selenium concentration in new growth wool was measured at wk 12, 24, 36, 48, and 60 (Table 5-5). Dietary Se level, Se source, time, dietary Se level × Se source, and dietary Se source × time affected ($P < 0.05$) wool Se. Wool Se ranged from 1.19 to 39.09 mg/kg and increased linearly ($P < 0.001$) as dietary Se increased. Wool Se from wethers receiving organic Se was often more than three-fold higher ($P < 0.001$) than from wethers receiving selenite Se at the same dietary level. Increased Se in hair has been reported in other livestock species. Kim and Mahan (2001) observed a linear response in the hair of pigs as Se in their diet was increased. Goehring et al. (1984b) reported a quadratic response in the hair of swine as dietary Se as sodium selenite was increased up to 20 mg/kg. Likewise, Perry et al. (1976) reported increased Se in the hair of feedlot steers as dietary selenite Se was increased. Cristaldi et al. (2004) reported a linear increase in the wool of wether sheep as dietary Se was increased. Those authors did not report a significant Se level × time interaction. However, wool Se of sheep, on the present study was affected by time and the interaction of Se source × time as wool Se continued to increase from wethers fed selenite Se and wool Se from wethers receiving organic Se increased then seemed to reach a peak around wk 48. This suggests that wool Se may reach a plateau when animals are fed high dietary concentrations of organic Se. Wool Se

concentrations in the present study were more than ten-fold higher than concentrations of 2 to 2.5 mg/kg in wool from wethers fed up to 10 mg/kg dietary Se as selenite (Cristaldi et al., in press), but never exceeded 40 mg/kg which is less than 45 mg/kg which was described as the Se concentration in hair of animals suffering from alkali disease (National Academy of Sciences [NAS], 1971).

Selenium concentrations in brain, diaphragm, heart, kidney, and loin muscle were affected ($P < 0.05$) by dietary Se level, Se source, and dietary Se × Se source interaction. Hoof Se concentration was affected by source ($P < 0.05$) and liver Se was affected ($P < 0.05$) by dietary Se level and dietary Se × Se source interaction, and tended to be affected ($P = 0.11$) by Se source. Selenium concentrations, on a DM basis, were highest in liver followed by kidney, heart, hoof, brain, loin, and diaphragm (Table 5-6). This pattern is similar to a ranking of Se concentrations in tissues of farm animals by (Combs and Combs, 1986) with the exception of liver and kidney being reversed. However, in animals fed Se at or below requirements, kidney generally has a higher concentration of Se than does the liver, but when dietary Se is increased, liver Se quickly becomes higher in Se than kidney. This supported by the work of Ewan et al. (1968) and the findings of Cristaldi et al. (2004) in sheep, and McDowell et al. (1977) in swine. Those authors reported higher liver Se than kidney Se when dietary Se was increased. Unlike minerals such as Zn and Mn, the status of Se is reflected in many tissues (McDowell, 2003). Brain Se concentrations ranged from 1.28 to 32.3 mg/kg and brain Se concentrations from wethers receiving organic Se were higher ($P < 0.001$) than brain Se from wethers receiving selenite Se. These results suggest that Se does cross the blood-brain barrier and that brain Se is influenced by dietary Se. Previous research using sheep supports our

findings of increased Se in brain as dietary Se is increased (Yeh et al., 1995;1997; Ouazzani et al., 1999). Diaphragm Se ranged from 0.82 to 26.34 mg/kg and tended to increase linearly ($P = 0.089$) as dietary Se increased. Diaphragm Se was higher ($P < 0.001$) in wethers receiving organic Se than from wethers receiving selenite Se. Heart Se ranged from 1.59 to 33.93 mg/kg and, like brain and diaphragm Se was higher ($P < 0.001$) in wethers receiving organic Se than from wethers receiving selenite Se. Selenium concentrations in the hoof tip ranged from 3.44 to 29.20 mg/kg and increased linearly as dietary Se increased ($P < 0.05$). Selenium concentrations of hoof tip taken from wethers receiving organic Se tended ($P = 0.07$) to be higher than from wethers receiving inorganic Se. Both Se sources produced hoof Se concentrations higher than 10 mg/kg which was previously reported in animals with alkali disease (NAS, 1971). Kidney Se tended ($P = 0.07$) to respond linearly to increased dietary Se and ranged from 8.43 to 77.61 mg/kg. Kidney Se concentrations from wethers receiving organic Se were higher ($P < 0.01$) than from wethers receiving selenite Se. Kidney Se concentrations from the present study are much higher than those reported by Maag and Glenn (1967) where death due to Se toxicosis was produced in 245 kg Hereford steers. However, the calves used by those authors received approximately 270 mg $Se \cdot steer^{-1} \cdot d^{-1}$ as sodium selenite and death was induced within 6 wk. Liver Se concentrations ranged from 2.66 to 132.73 mg/kg and increased linearly ($P < 0.001$) as dietary Se level increased. Selenium concentrations in liver from wethers receiving organic Se were not different ($P = 0.34$) than liver Se concentrations from wethers receiving selenite Se. Selenium concentrations in the loin muscle (psoas major), which is often consumed by mankind ranged from 0.71 to 26.87 mg/kg and tended ($P = 0.12$) to increase linearly as dietary Se

was increased. Organic Se was more effective ($P < 0.001$) at increasing Se concentrations in edible tissue than was selenite Se. As daily intake of Se by humans declines in some parts of the world, increasing the Se content of foods for human consumption by manipulating source and level of Se supplementation to livestock is now of interest to food scientists. Givens et al. (2004) suggested that the Se content of cows' milk could be increased through the use of Se yeast as the supplemental form of Se to dairy cows. Our findings indicate that Se content of muscle and organ tissue can be influenced by source and level of Se supplementation to food animals. In general, Se concentrations of brain, heart, kidney, liver, and muscle were much higher than those reported in studies with cattle (Maag and Glenn, 1967) and sheep (Glenn et al., 1964c). Deaths due to Se toxicosis were induced in both species. In contrast, Se death due to Se toxicosis was never produced during our study. It is important to note that in the two previous studies that animals were fed Se at higher levels and for a shorter period of time. Our findings further agree with Smith et al. (1937) who found that the effects of continued dosing of Se were cumulative and that Se from organic sources was accumulated in higher quantities in tissues than Se from inorganic sources.

Most of the heart, diaphragm, loin, liver, and kidney tissues subjected to histopathological evaluation were free from pathological changes. The findings of lymphocytes in the portal triads were deemed to be a background finding and insignificant. Three instances of vacuolic degeneration associated with the cytoplasm of hepatocytes suggesting fatty degeneration were noted. However, no pattern associating abnormal pathology to either dietary Se level or source could be established. Therefore, lesions could not be definitively linked to treatment and could have been metabolic in

nature. Cristaldi et al. (2004) found no abnormalities after microscopic evaluation of heart, liver, kidney, diaphragm, and muscle from wethers consuming up 10 mg/kg Se for one yr. Examination of kidney, heart, and liver tissues by transmission electron microscopy did not reveal any apparent changes in cell structure as related to Se toxicosis and no differences in tissue cells from controls and wethers receiving 20, 30, or 40 mg/kg Se were observed.

Concentrations of albumin and activities of Alk phos, ALT, GGT, AST, and CK in serum collected at the termination of the experiment were, in general, in or below the normal range for adult sheep (Table 5-7). In instances of Se toxicosis, the activities of these enzymes would have been increased due to tissue necrosis. Our observations agree with those reported by Cristaldi et al. (2004) as albumin and enzyme activities in wether sheep after receiving up to 10 mg/kg Se were in the normal ranges. The lack of elevated enzymes, which are suggestive of tissue necrosis, further indicates that the wethers on our study were not suffering from Se toxicosis.

Throughout this 60-wk experiment clinical signs of Se toxicosis (e.g., lameness, wool loss, and abnormal hoof growth) were not observed, though serum and whole blood Se concentrations were frequently higher than those described in livestock diagnosed with hyperselenosis. However, wool Se concentrations from wethers on our study never reached the levels previously reported in the hair of livestock suffering from alkali disease. Loin muscle and diaphragm showed no gross lesions at slaughter and no abnormality was observed with microscopic evaluation. Abnormal pathology in the kidney, heart, and liver was rare and could, in each case, be attributed to a cause other than Se toxicosis. No pale focal areas were observed in the myocardium, though

previous research (Glenn et al., 1964b; Smyth et al., 1990) has shown the heart to be the target organ in instances of Se toxicosis. No abnormalities were prevalent enough to establish a treatment-related pattern and no wethers receiving the maximum level of dietary Se on our study (40 mg/kg) showed any abnormal tissue lesions. Further evaluation of kidney, heart, and liver using transmission electron microscopy also revealed no cellular abnormalities and enzymes, suggestive of tissue necrosis, were in or below normal ranges at the termination of the experiment. Without the presence of tissue damage and clinical signs, it seems that Se toxicosis was not induced in wether sheep fed up to 40 mg/kg dietary Se as Se yeast or sodium selenite. However, Se concentrations in serum, blood, wool, and tissues from wethers receiving organic Se indicate that Se toxicity is dependent on Se source and that much inorganic dietary Se is reduced to insoluble forms. The work of Cousins and Cairney (1961), Whanger et al. (1968), and Koenig et al. (1997) support our findings.

Implications

The current estimate of the maximum tolerable dietary level of selenium for sheep (2 mg/kg) seems to be grossly underestimated. Selenium, whether organic or inorganic in form, can be fed as high as 40 mg/kg for up to 60 wk without inducing Se toxicosis. Previously the range between optimal and toxic levels of selenium was reported as narrow; however data from the present study would suggest that the range is relatively wide. Increasing dietary selenium level, regardless of source, is an effective means of increasing selenium in blood and tissues. Organic selenium is more greatly accumulated by organs and tissues. Manipulation of dietary selenium source and level is an effective way to change the selenium content of animal tissues commonly consumed by mankind.

Summary

The objectives of this 60-wk experiment were to evaluate and compare effects of feeding Se as sodium selenite or Se yeast at high dietary levels on serum, whole blood, wool, and tissue Se concentrations in wether sheep and determine maximum tolerable level of Se. Twenty-eight, 2-yr-old, Rambouillet-crossbred wethers (62.3 ± 8.5 kg initial BW) were utilized in a 2 × 4 factorial arrangement with 0.2, 20, 30, and 40 mg/kg dietary Se (as-fed) from sodium selenite or Se yeast added to a corn-soybean meal basal diet. Wethers were weighed at 8-wk intervals, serum Se, whole blood Se, and wool Se were measured every 12 wk, and samples of brain, diaphragm, heart, hoof, kidney, liver, and loin muscle and serum samples for evaluation of albumin and enzyme activities were collected at the termination of the experiment. Wether BW was affected by dietary Se level ($P < 0.05$), source of dietary Se ($P < 0.05$), and time ($P < 0.05$). Average BW decreased linearly ($P < 0.10$) as dietary Se level increased, though most wethers gained BW. Serum Se, whole blood Se, and wool Se concentrations were affected ($P < 0.05$) by dietary level of Se and source of Se. Serum Se and whole blood Se ranged from 110 to 3922 μg/L and 392 to 6259 μg/L, respectively, and increased in a quadratic fashion as dietary Se level increased ($P < 0.05$) and wool Se ranged from 1.19 to 39.09 mg/kg and responded linearly ($P < 0.05$) to increased dietary Se. Serum Se, whole blood Se, and wool Se concentrations from wethers receiving organic Se were higher ($P < 0.01$) than those from wethers receiving inorganic Se. Selenium concentrations in brain, diaphragm, heart, hoof, kidney, liver, and loin muscle were affected ($P < 0.05$) by dietary Se level, with higher Se concentrations generally observed in tissues from wethers receiving organic Se. Though Se concentrations in serum, blood, wool, and major organs at most times exceeded concentrations previously reported in livestock suffering from Se

toxicosis, a pattern of clinical signs of Se toxicosis was not observed in this experiment. Microscopic evaluation of liver, kidney, diaphragm, heart, and psoas major muscle did not reveal definitive evidence of Se toxicosis in wethers on any dietary Se treatment. Wethers under our experimental conditions tolerated up to 40 mg/kg dietary Se for 60 wk, though differences in Se source were observed. Contrary to previous thought, the range between optimal and toxic dietary level of Se is not narrow. The maximum tolerable level of dietary Se, regardless of source, is much higher than the current estimate of 2 mg/kg.

Table 5-1. Diet composition (as-fed) for Se supplemented wethers[a]

Ingredient	% as-fed
Ground yellow corn	58.00
Cottonseed hulls	30.00
Soybean meal (47.5% CP)	6.50
Soybean oil	3.00
Trace mineral mix[b]	1.00
Ground limestone	1.00
Ammonium chloride	0.50
Vitamins A & D	—[c]

[a]Selenium levels in diet (as analyzed): 0.48, 20.48, 30.86, and 38.10 mg/kg for Se levels 0.2, 20, 30, and 40 mg/kg from sodium selenite, respectively; 0.54, 20.26, 30.71 and 37.65 mg/kg for Se levels 0.2, 20, 30, and 40 mg/kg Se from Se yeast, respectively

[b]Trace mineral mixture supplied between 96.5% and 98.5% NaCl, and provided per kg of diet: 1.0 mg Co (as carbonate), 5.0 mg Cu (as oxide), 0.7 mg I (as iodate), 35 mg Fe (as oxide), 25 mg Mn (as oxide), and 35 mg Zn (as oxide).

[c]Provided per kg of diet: 5,000 IU of Vitamin A and 500 IU of Vitamin D_3.

Table 5-2. Effects of four dietary levels of Se as sodium selenite or Se yeast on BW of wethers[a]

	Se source								
	Sodium selenite				Se yeast				
	Dietary Se level, mg/kg								
	0.2	20	30	40	0.2	20	30	40	
Week	Wether BW, kg								SEM
0	61.2	65.0	65.8	58.9	57.0	55.5	67.9	64.3	4.5
8	59.1	63.6	59.8	49.3	56.8	51.5	54.3	50.6	4.5
16	61.8	68.2	57.9	48.2	57.9	55.1	54.8	50.0	5.7[e]
24	65.1	70.0	59.2	52.4	60.0	57.3	53.3	48.2	7.5[e]
32	70.9	76.7	63.6	56.1	68.2	59.5	51.1	52.7	9.0[e]
40	70.5	61.8	61.8	57.3	74.7	51.8	47.3	50.9	8.4
48	77.6	81.8	69.8	62.9	70.5	65.5	38.6	54.5	9.0[c]
60	83.3	85.6	76.5	67.9	78.2	61.8	50.2	54.5	10.4
Avg	68.7	71.6	64.3	56.6	65.4	57.3	52.2	53.2	7.2[bcde]

[a]Data represent least squares means and pooled SE.

[b]Dietary Se level response ($P < 0.05$).

[c]Selenium source response ($P < 0.05$).

[d]Time response ($P < 0.05$).

[e]Dietary Se level linear response ($P < 0.10$).

Table 5-3. Serum Se concentrations of wethers fed four dietary levels of Se as sodium selenite or Se yeast[a]

	Se source							
	Sodium selenite				Se yeast			
	Dietary Se level, mg/kg							
	0.2	20	30	40	0.2	20	30	40	
Week	Serum Se, µg/L								SEM
12	157	548	788	1000	412	2583	3210	2458	249[bcdefg]
24	130	1683	1487	1724	354	2639	3922	1585	826[bcf]
36	444	851	960	1083	540	3283	2086	1409	250[bcdf]
48	110	822	1219	1496	292	2428	2076	1831	253[bcef]
60	119	610	886	1250	424	1699	2712	2549	331[bcef]
Avg	192	903	1068	1311	404	2526	2801	1966	395[bcdf]

[a]Data represent least squares means and pooled SE.
[b]Dietary Se level response ($P < 0.05$).
[c]Selenium source response ($P < 0.05$).
[d]Dietary Se level × Se source interaction ($P < 0.05$).
[e]Dietary Se level linear response ($P < 0.05$).
[f]Dietary Se level quadratic response ($P < 0.05$).

Table 5-4. Whole blood concentrations of wethers fed four dietary levels of Se as sodium selenite or Se yeast[a]

	Se source							
	Sodium selenite				Se yeast			
	Dietary Se level, mg/kg							
	0.2	20	30	40	0.2	20	30	40	
Week	Whole blood Se, µg/L								SEM
12	392	1172	1484	1315	1183	4344	4290	5484	475[bcde]
24	420	1551	2228	2353	1661	4521	6259	5780	415[bce]
36	1004	1021	1708	2406	1549	5018	4841	1972	635[c]
48	393	1772	1977	2416	1068	5061	5220	4929	298[bcde]
60	402	1258	1621	2043	1500	1759	3629	4914	435[bce]
Avg	522	1355	1804	2107	1392	4028	4803	4408	534[bcdf]

[a]Data represent least squares means and pooled SE.
[b]Dietary Se level response ($P < 0.05$).
[c]Selenium source response ($P < 0.05$).
[d]Dietary Se level × Se source interaction ($P < 0.05$).
[e]Dietary Se level linear response ($P < 0.10$).
[f]Dietary Se level quadratic response ($P < 0.05$).

Table 5-5. Wool Se concentrations of wethers fed four dietary levels of Se as sodium selenite or Se yeast[a]

	Se source								
	Sodium selenite				Se yeast				
	Dietary Se level, mg/kg								
	0.2	20	30	40	0.2	20	30	40	
Week	Wool Se, mg/kg								SEM
12	1.37	3.27	6.69	4.15	3.78	12.67	21.09	24.26	3.80[bce]
24	1.47	3.57	5.72	11.92	7.04	31.58	35.69	37.30	2.87[bcd]
36	1.68	6.02	9.85	10.85	5.70	18.99	22.79	21.29	4.72[ce]
48	1.19	3.15	5.64	7.23	6.39	24.81	39.09	29.65	2.22[bcd]
60	1.29	3.90	5.01	6.23	4.38	23.22	25.65	25.99	2.01[bcd]
Avg	1.40	3.98	6.58	8.08	5.46	22.25	28.87	27.70	3.38[bcdefg]

[a]Data represent least squares means and pooled SE.
[b]Dietary Se level response ($P < 0.05$).
[c]Selenium source response ($P < 0.05$).
[d]Dietary Se level × Se source interaction ($P < 0.05$).
[e]Dietary Se level linear response ($P < 0.10$).
[f]Time response ($P < 0.05$).
[g]Time × Se source interaction ($P < 0.05$).

Table 5-6. Effects of four dietary levels of Se as sodium selenite or Se yeast on tissue Se of wethers[a]

	Se source								
	Sodium selenite				Se yeast				
	Dietary Se level, mg/kg								
	0.2	20	30	40	0.2	20	30	40	
Tissue	Se concentration, mg/kg								SEM
Brain	1.28	4.22	4.74	6.87	6.12	21.90	32.30	18.71	0.99[bcd]
Diaphragm	0.82	4.74	3.33	7.81	5.28	10.30	26.34	20.71	2.69[bcde]
Heart	1.59	3.80	5.13	6.23	6.35	23.77	28.71	33.93	2.43[bcd]
Hoof	3.44	8.79	9.68	13.78	6.26	12.53	29.20	23.66	5.52[ce]
Kidney	8.43	19.94	27.93	27.89	22.26	33.96	77.61	36.28	6.87[bcde]
Liver	2.66	31.72	41.42	78.18	15.67	23.42	132.73	41.24	18.17[bde]
Loin	0.71	3.13	4.41	5.13	5.73	14.69	23.51	26.87	1.05[bcd]

[a]Data represent least squares means and pooled SE.
[b]Dietary Se level response ($P < 0.05$).
[c]Selenium source response ($P < 0.05$).
[d]Dietary Se level × Se source interaction ($P < 0.05$).
[e]Dietary Se level linear response ($P < 0.10$).

Table 5-7. Amount of albumin and tissue enzyme activities present in serum of Se supplemented wethers[a,b]

	Se source								
	Sodium selenite				Se yeast				
	Dietary Se level, mg/kg								
	0.2	20	30	40	0.2	20	30	40	Normal
Enzyme	Se concentration, mg/kg								range
Albumin, g/dL	3.0	3.0	2.9	2.7	2.8	2.0	2.5	2.9	2.4 - 4.0
Alk Phos, IU/L	127.3	141.7	128.8	153.5	105.7	32.0	50.5	172.0	68 - 387
ALT, IU/L	12.0	12.0	10.8	9.3	9.7	3.0	2.5	6.0	11 - 40
AST, IU/L	81.7	100.7	108.8	81.8	94.7	83.0	65.0	53.0	60 - 280
GGT, IU/L	49.3	51.7	61.8	60.5	54.3	42.0	46.5	59.0	15 - 60
CK, IU/L	123.7	139.7	75.0	103.0	109.0	48.0	50.0	51.0	00 - 584

[a]Serum sample collected at wk 60.

[b]GGT and CK ranges were established by University of Florida Veterinary Teaching Hospital.

CHAPTER 6
EFFECTS OF FORM OF PARENTERAL OR DIETARY SELENIUM SUPPLEMENTATION ON BODY WEIGHT AND BLOOD, LIVER, AND MILK CONCENTRATIONS IN BEEF COWS

Introduction

Many areas of the United States have selenium deficient soils (McDowell, 2003) and may produce forages and grains which are unable to provide adequate Se to livestock. Selenium deficient brood cows may give birth to calves which are stillborn, premature, weak, or afflicted with nutritional muscular degeneration (Maas, 1983; Corah and Ives, 1991). Likewise, even with adequate blood Se at birth, calves suckling Se deficient dams are susceptible to becoming Se deficient (Pehrson et al., 1999). Without adequate dietary or parenteral Se supplementation, brood cows may suffer from infertility, retained placentas, ovarian cysts, metritis, silent estrus periods, and/or poor weight gains (Dargatz and Ross, 1996).

In cattle, it has been well established that Se crosses the placenta (Koller et al., 1984; Van Saun et al., 1989), that dietary Se is transferred to milk (Conrad and Moxon, 1979), and that positive correlations exist between blood Se of cows and blood Se of their calves (Kincaid and Hodgson, 1989; Enjalbert et al., 1999; Pehrson et al., 1999). The chemical form of Se affects its metabolism and previous research has shown differences in blood, milk, and liver Se concentrations due to form (organic vs inorganic) of supplemental Se (Knowles et al., 1999; Gunter et al., 2003; Valle et al., 2002).

Selenium is often supplemented as sodium selenite and included in free-choice livestock mineral mixtures. However, Se may be supplemented through subcutaneous

injection of barium selenate, sodium selenate or sodium selenite and, in ruminants, with slow-release, long lasting ruminal Se boluses or pellets. With the recent Food and Drug Administration approval of Se yeast for use in ruminant diets, livestock producers now have more choices of form and method of Se supplementation. The objective of this experiment was to evaluate and compare effects of form and method of Se supplementation on blood, liver, and milk Se concentrations in beef cows.

Materials and Methods

All animal procedures were conducted within the guidelines of the University of Florida Institutional Animal Care and Use Committee. Animals were housed at the University of Florida Boston Farm-Santa Fe Beef Unit located in Northern Alachua County, Florida. On August 6, 2002, 43 Angus cows, aged 2-3 yr, (mean age = 2.67 yr) were palpated to diagnose pregnancy and estimate d in gestation. All cows were determined pregnant and gestation estimates ranged from 115 to 130 d. Each animal received a chemically altered modified live 4-way viral + vibriosis and leptospirosis vaccination (Cattlemaster 4+VL-5; Pfizer Animal Health, Exton, PA) and fly control (Permectrin 10% EC pour-on; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) according to manufacturer directions. Cows were weighed (average initial BW = 417 ± 46 kg), stratified by age and assigned to one of five treatment groups for a 365 d study. The five treatments were 1) no Se supplementation, control group, 2) one subcutaneous injection of 9 mL (50 mg Se·mL^{-1}) barium selenate (Deposel Multidose; Novartis New Zealand, Ltd., Auckland, NZ) at the initiation of the experiment, 3) three subcutaneous injections of 5 mL (5 mg Se·mL^{-1}) of sodium selenite + 68 IU vitamin E as dl-alpha tocopheryl acetate (Mu-Se; Schering-Plough Animal Health, Union, NJ), one at the initiation of the experiment and one every four mo thereafter, 4) free-choice access to a

mineral mixture containing 26 mg Se/kg as sodium selenite (Southeastern Minerals, Inc., Bainbridge, GA), or 5) free-choice access to a mineral mixture containing 26 mg Se/kg as Se yeast (Sel-Plex; Alltech, Inc, Nicholasville, KY). All cows grazed bahiagrass (*Paspalum notatum*) pastures and were supplemented with bahiagrass (*Paspalum notatum*) hay, molasses-based liquid supplement ad libitum, and whole cottonseed and pelleted citrus pulp at rates of 0.68 $kg \cdot cow^{-1} \cdot d^{-1}$ and 1.81 $kg \cdot cow^{-1} \cdot d^{-1}$, respectively, from November, 2002 through March, 2003.

Treatment groups receiving no Se or injectable Se were housed together and had access to a free-choice mineral mixture containing no Se (Table 6-1). Cows receiving Se via free-choice mineral mixtures were housed in separate groups and had access to the same mineral mixture with added Se as sodium selenite or Se yeast for treatments 4 and 5, respectively (Table 6-1). All free-choice mineral mixtures were offered in wooden mineral feeders and protected from rain.

Blood samples for plasma analyses were collected via jugular venipuncture into 10-mL heparinized tubes (Vacutainer; Becton-Dickinson, Franklin Lakes, NJ) at the initiation of the study (d 0) and at d 365. Calving occurred over a 24 d span between December 31, 2002 and January 23, 2003 and whole blood samples were collected in the same manner from all cows immediately after parturition and at 30, 90, and 205 d postpartum. A colostrum or milk sample was also collected on those days into a 15-mL plastic centrifuge tube. Forty-one calves, 24 male, 17 female, were born alive and unassisted. One cow in the free-choice selenite group had a stillbirth and one control cow aborted very early in the experiment and both were removed from the study. Liver biopsies were performed on all cows using the technique described by Chapman et al.

(1963) on d 0 and d 365. After each collection, liver, whole blood, and/or milk samples were placed on ice, transported to the University of Florida Animal Nutrition Laboratory, and then frozen (0°C) until analyses. Blood collected for plasma analyses was centrifuged at 700 × g for 25 min; following centrifugation, plasma was frozen for later analyses. Cows were weighed again at the cessation of the experiment and change in BW was calculated.

Pastures were sampled in October 2002 pre-calving, March 2003 post-calving, and August 2003 at weaning. Likewise, hay, molasses-based liquid supplement, whole cottonseed, and pelleted citrus pulp were sampled during winter supplementation. Whole blood, liver, plasma, milk, and all feedstuffs were analyzed for Se concentration using a fluorometric method described by Whetter and Ullrey (1978). To help ensure reliability of the analytical method, a certified standard (National Bureau of Standards Bovine Liver SRM-1577a; U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) was frequently analyzed.

Effects of treatment on change in body weight were analyzed using PROC MIXED in SAS (SAS for Windows 8e; SAS Inst., Inc., Cary, NC) in a completely randomized design with a diagonal covariance structure. Contrast statements were used to compare means as described by Littell et al. (1998; 2000). PROC MIXED was also used to analyze effects of treatment, d, and the interaction of treatment × d on body weight, whole blood Se, milk Se, plasma Se, and liver Se as repeated measures with a spatial power covariance structure with respect to d and subplot of animal nested within treatment. Contrast statements were written to determine differences in means for different sampling d. PROC CORR was used to determine correlations of milk Se to

whole blood Se from parturition to 205 d postpartum and to determine the correlation of plasma Se to liver Se.

Results

Selenium concentration of pasture and hay for all groups averaged 0.071 ± 0.014 and 0.045 mg/kg DM basis, respectively. Crude protein was 12.57% for hay and 12.79% ± 1.76 for standing forage. Forage CP did not differ ($P = 0.92$) among pastures during the study. Whole cottonseed, pelleted citrus pulp, and liquid supplement analyzed 0.002, 0.002, and 0.744 mg Se/kg, respectively. Free-choice mineral consumption was monitored and recorded. Mineral consumptions and amount of Se via injection are summarized in Table 6-2. All free-choice mineral mixtures were formulated for an expected intake of 85-113 $g \cdot cow^{-1} \cdot d^{-1}$. However, only the cows on the Se yeast treatment had mineral consumption (85.5 $g \cdot cow^{-1} \cdot d^{-1}$) in this range (Table 6-2). Treatment groups receiving free-choice minerals with no Se consumed 62.19 $g \cdot cow^{-1} \cdot d^{-1}$ and cows receiving free-choice minerals with sodium selenite consumed 41.5 $g \cdot cow^{-1} \cdot d^{-1}$.

There was an effect of d and treatment × d ($P < 0.001$) on cow BW. Initial BW of cows did not differ ($P > 0.40$) among treatment groups (Table 6-3) and all cows gained weight ($P < 0.03$). At the end of study (d 365), cows that received Sel-Plex were heavier ($P < 0.05$) than controls, and cows receiving either injectable Se source. Cows from the free-choice selenite treatment were heavier ($P < 0.05$) than those receiving Deposel and tended ($P = 0.085$) to be heavier than controls and Mu-Se treated cows. Form of Se supplementation had an effect ($P < 0.001$) on change in cows' BW (Figure 6-1). Both free-choice treatment groups were similar and had a greater increase in BW ($P < 0.05$) than did control and the injectable Se treated groups. Control, Mu-Se, and Deposel

treated cows had similar changes in BW and cows receiving Mu-Se tended ($P = 0.07$) to gain more weight than cows receiving Deposel.

Cow whole blood Se concentrations at intervals postpartum are summarized in Table 6-4. Significant effects of treatment ($P < 0.001$), d ($P < 0.001$), and treatment × d ($P = 0.013$) were observed. At parturition, whole blood Se concentrations from cows receiving Deposel or Sel-Plex were higher ($P < 0.05$) than whole blood Se from controls and cows receiving Mu-Se or free-choice selenite. Also, Mu-Se tended ($P = 0.13$) to produce blood Se higher than control. At 30 d postpartum, no differences ($P > 0.05$) in whole blood Se were observed among Deposel, Mu-Se, and free-choice selenite treatment groups. Cows receiving Deposel had higher ($P < 0.05$) blood Se than controls and tended ($P = 0.08$) to have higher blood Se than cows receiving Mu-Se. Cows receiving Sel-Plex tended to have higher ($P = 0.053$) blood Se concentrations than did controls and cows receiving any other form of Se supplementation at 30 d postpartum.

At 90 d postpartum, Sel-Plex produced blood Se concentrations higher ($P < 0.05$) than did free-choice selenite, Mu-Se, or no Se supplementation. Deposel treated cows had blood Se higher ($P < 0.05$) than Mu-Se treated cows and the controls and tended to be higher ($P = 0.14$) in blood Se than cows receiving free-choice selenite. Blood Se from the free-choice selenite group was similar ($P = 0.46$) to that from Mu-Se and higher ($P < 0.001$) than from controls. Again, at 205 d postpartum, which coincided with weaning of calves, Sel-Plex treated cows had blood Se higher ($P < 0.05$) than cows from all other treatment groups. Free-choice selenite and Deposel treated cows had similar ($P = 0.92$) blood Se, which was higher ($P < 0.05$) than blood Se from controls and the Mu-Se group. A third order polynomial response ($P = 0.001$) was observed, as overall, whole blood Se

values increased from d 0 to d 30 postpartum and decreased from d 30 to d 205 postpartum.

Effects of treatment and d ($P < 0.001$) were observed in Se concentration of milk collected at the same postpartum intervals as whole blood (Table 6-5). Cows receiving Sel-Plex had higher ($P < 0.05$) Se concentrations in post-suckled colostrum than did cows receiving all other treatments. Colostrum Se was similar ($P > 0.54$) from control, Deposel, Mu-Se and free-choice selenite treated cows. No significant differences in milk Se were observed at 30 d postpartum, however, Sel-Plex treated cows tended ($P = 0.13$) to produce milk with higher Se concentration than did cows that received Mu-Se. At 90 d postpartum, no differences or tendencies were observed in milk Se among treatment groups ($P > 0.28$). Selenium in milk collected at 205 d postpartum, was similar ($P > 0.50$) among control, Mu-Se, Deposel, and free-choice selenite treatments. Cows receiving Sel-Plex produced higher ($P < 0.01$) milk Se than cows receiving any other form of Se supplementation. Milk Se from all treatment groups decreased quadratically ($P < 0.001$) from parturition to 205 d postpartum. Selenium concentrations in whole blood and milk collected at parturition and 30, 90, and 205 were positively correlated ($P < 0.01$; $r = 0.25$).

Plasma Se concentrations were evaluated at d 0 and at d 365 (Table 6-6). Treatment and treatment × d had significant effects ($P < 0.001$) on plasma Se. At d 0, all cows had similar plasma Se (52 to 62 μg/L). At the end of the study, only cows from the control and Mu-Se treatments had similar ($P = 0.32$) plasma Se. Plasma Se concentrations in Sel-Plex treated cows were higher ($P < 0.005$) than from cows receiving any other treatment. Free-choice selenite produced higher ($P < 0.012$) plasma

Se than did control or Mu-Se, and Deposel produced higher ($P < 0.001$) plasma Se than did control, free-choice selenite, or Mu-Se. After one yr, only cows receiving Sel-Plex had increased ($P < 0.001$) plasma Se. Deposel tended to increase ($P = 0.13$) plasma Se, while plasma Se decreased ($P < 0.001$) in control and Mu-Se treated cattle, and plasma Se concentrations in cows receiving Se as free-choice selenite were unchanged ($P = 0.69$).

Liver from biopsies at d 0 and d 365 was evaluated for Se concentration (Table 6-7). As with plasma Se, treatment and treatment × d had significant effects ($P < 0.001$). Liver Se (946 to 1136 μg/kg) did not differ among treatment groups at d 0 ($P > 0.31$). However, at d 365, liver Se from Sel-Plex treated animals was higher ($P < 0.02$) than from animals on all other treatments. Liver Se concentrations from cows receiving Se from Deposel or free-choice selenite were similar, ($P = 0.21$) and both were higher ($P < 0.05$) than those of controls and cows receiving Mu-Se. At the end of this study, liver Se had increased ($P < 0.001$) in cows receiving Sel-Plex. Cows receiving Mu-Se had decreased ($P < 0.01$) liver Se and liver Se tended to decrease ($P = 0.07$) in controls. Liver Se remained unchanged ($P = 0.48$; 0.73) in cows receiving Deposel and free-choice mineral with sodium selenite, respectively. Liver and plasma Se concentrations were highly correlated ($r = 0.71$; $P < 0.001$).

Discussion

No differences in cow BW were observed among the control, Mu-Se, and Deposel treatment groups. However, BW of cows receiving Se supplementation from Se yeast or sodium selenite in free-choice minerals was higher than controls and cows receiving injectable Se. Gunter et al. (2003) compared effects of Se yeast and sodium selenite on cow performance and found no differences in BW between cows that received Se yeast or

sodium selenite. Likewise, those authors did not report a difference in BW of Se supplemented cows vs unsupplemented controls. Likewise, Awadeh et al. (1998b) reported no differences in the BW of Angus and crossbred cows supplemented with Se yeast and various levels of sodium selenite. Bruce (1997) also reported no differences in BW of cows in Alaska regardless of Se supplementation. Cow age, when reported, indicated that cows used in previous studies were mature and may have had less potential for growth than the younger cows utilized in our study.

At calving, cows receiving Se via Deposel or Sel-Plex had higher whole blood Se than did cows receiving no Se, Mu-Se, or selenite in free-choice minerals. Whole blood Se measured at 30 and 90 d postpartum followed a pattern similar with respect to treatment to whole blood Se at calving. Deposel and Sel-Plex produced similar and consistently higher whole blood Se than sodium selenite or no Se supplementation. In an Arkansas study, Gunter et al. (2003) found higher whole blood Se at calving in cows receiving Se yeast than in cows receiving sodium selenite. Knowles et al. (1999) reported whole blood Se in cows supplemented with Se yeast to be similar to, or higher than from cows receiving sodium selenate in a 19-wk study during mid-lactation. However, Awadeh et al. (1998b) did not report a difference in whole blood Se when comparing dietary Se at equal concentrations as Se yeast or sodium selenite. Podoll et al. (1992) did report a slight increase in serum Se for selenate vs selenite fed to lactating Holstein cows and Awadeh et al. (1998a) reported an increase in whole blood Se at calving for Se yeast vs selenite when added to free-choice salt at 60 mg/kg. Increases in whole blood Se from Deposel over either selenite form of Se are further supported by the work of Henry et al. (1988) as those authors reported that selenate was more available to

ruminants than selenite. From d 90 to d 205 postpartum, whole blood Se decreased in controls and cows receiving Mu-Se, and both were below the adequate whole blood Se level (> 100μg/L) as defined by Gerloff (1992) and Dargatz and Ross (1996). Cows receiving Se from Deposel or either free-choice mineral mix maintained whole blood Se above the adequate level from parturition to 205 d postpartum. At d 205 postpartum, all controls and 89% of cows receiving Mu-Se had whole blood Se below the adequate level. Pehrson et al. (1999) reported whole blood Se concentrations above adequacy in Hereford cows while nursing calves, and observed no differences between organic and inorganic forms of Se supplementation.

Differences in milk Se concentrations from this study, agree with findings of Valle et al. (2002) as, in both experiments, cows supplemented with Se yeast consistently produced milk Se higher than or similar to cows receiving selenite or selenate. Likewise, colostrum Se from cows receiving organic Se was higher than in controls and inorganic Se treated cows. Ortman and Pehrson (1999) reported milk Se values more than 100% higher from Swedish dairy cows receiving Se yeast than from cows receiving inorganic or no Se supplementation. Also, beef cows supplemented with Se yeast were consistently higher in milk Se at calving and at approximately 35 d postpartum than cows receiving sodium selenite (Pehrson et al., 1999). Results have been similar in other species; Mahan (2000) reported higher milk Se at seven and 14 d postpartum from sows receiving organic vs inorganic Se fed at the same levels. In our study, though differences were observed in blood Se from selenate vs selenite, no differences in milk or colostrum Se were observed between the two inorganic sources. Our findings agree with those of Ortman and Pehrson (1999) and support their statement that there is no difference in effect of selenate

and selenite on milk Se concentrations. The overall depression in whole blood and milk Se concentrations at 90 d postpartum supports the idea set forth by Pehrson et al. (1999) and supported by the work of Valle et al. (2002), that the increase in consumption of lush, growing forage increases oxidative stress on the animals. This stress is likely due to increased unsaturated fatty acids, such as linolenic acid, and thus, Se content of milk and whole blood was decreased due to the cows need for antioxidants. Ninety d postpartum on our study was April 8th. In Florida and much of the southeastern U.S., cattle will consume more fresh grass than hay around that date.

Sel-Plex supplemented cows had higher liver Se at the end of our study than did cows receiving any other treatment. Sel-Plex produced liver Se concentrations up to three-fold higher than Mu-Se. Such dramatic increases and differences in liver Se among treatments after one yr reported by Valle et al. (2002) and are similar to ours, as Se yeast treated cows had liver Se higher than cows from control or inorganic Se treatments. Likewise, those authors reported Deposel treated cows as having intermediate in liver Se concentrations. Deposel performed similarly in the present study, and liver Se concentrations from controls and Mu-Se decreased in both experiments. All cows receiving Mu-Se and 83% of controls had liver Se concentrations below the adequate level (> 1200 μg/kg). Liver and plasma Se concentrations were highly correlated and followed a similar pattern with respect to increases and decreases over the course of this study. Plasma Se concentrations and differences among treatments were, again, similar to those reported by Valle et al. (2002). Control cows, cows receiving Mu-Se, and cows receiving free-choice selenite had lower plasma Se after one yr, whereas Deposel and Se-Plex treated cows were able to maintain or increase plasma Se, respectively. At the

termination of the experiment, 100% of cows supplemented with selenite, free-choice or injectable, and cows receiving no supplemental Se had plasma Se concentrations below the critical level of > 70 μg/L.

Implications

Organic forms of selenium supplemented in free-choice mineral mixtures and the selenate form of selenium as an injection were able to increase or maintain selenium levels in plasma and liver of beef cows. Either of these forms of selenium supplementation may be effective for open or gestating beef cows. However, grazing beef cows receiving no selenium supplementation or selenium as injectable sodium selenite had inadequate plasma and liver selenium. During times of stress such as calving and lactation, organic selenium was superior to other forms of supplementation in maintaining adequate blood selenium and milk selenium adequate for nursing calves.

Summary

In a 365 d study, the effects of chemical form and method of Se supplementation on blood, milk, and tissue Se in grazing beef cows were evaluated and compared. Forty-three Angus cows (115-130 d gestation) were randomly assigned to one of five groups and received either no Se supplementation (control), one 5-mL sodium selenite injection s.c. every four mo, one 9-mL barium selenate injection s.c. at the initiation of the study, or free-choice mineral mixtures containing 26 mg/kg Se as sodium selenite or Se yeast. Cows grazed bahiagrass (*Paspalum notatum*) pastures, received routine dietary supplementation, and calved mostly in early January 2003. Body weight, plasma Se, and liver Se were measured at d 0 and d 365. Whole blood and milk samples were taken at calving and 30, 90, and 205 d postpartum. Cows receiving Se in free-choice minerals were heavier and had a greater increase in BW at d 365 ($P < 0.05$) than cows receiving all

other treatments. Plasma Se and liver Se concentrations were not initially different. At d 365, plasma Se in cows receiving Se yeast was higher ($P < 0.05$) at 90 μg/L than from all other treatments. Injectable selenate was intermediate and produced higher plasma Se than control and both forms of selenite. Liver Se at d 365 was adequate (> 1200 μg/kg) and higher ($P < 0.05$) in Se yeast treated cows than all others. Cows receiving injectable selenate also had adequate liver Se concentrations that were higher ($P < 0.05$) than the inadequate levels from control, free-choice selenite and injectable selenite. Whole blood Se was adequate (> 100 μg/L) for all treatment groups at calving, 30 and 90 d postpartum. At 205 d postpartum, cows receiving injectable selenate and both free-choice treatments were adequate in whole blood Se, while controls and cows receiving injectable selenite had inadequate whole blood Se. Cows receiving Se yeast produced higher ($P < 0.05$) colostrum Se than all other treatments. No differences were observed in milk Se at 30 and 90 d postpartum among treatment groups, however, both free-choice and the injectable selenate treated cows had milk Se numerically higher than controls and cows receiving injectable selenite. At weaning (205 d postpartum), cows receiving Se yeast had at least two-fold higher ($P < 0.05$) milk Se than cows receiving other treatments. Selenium supplementation with organic or inorganic Se via free-choice minerals or injectable selenate maintained adequate Se concentrations in whole blood, plasma, and liver. Inorganic Se was limited in its ability to increase milk Se, whereas Se yeast increased milk Se at parturition and at weaning.

Table 6-1. Composition of mineral mixtures offered free-choice to brood cows

Component	Free-choice mineral mixtures		
	No Se[a]	Sodium selenite[b]	Se yeast[c]
	%, as-fed		
Calcium	18.73	18.73	18.49
Phosphorus	8.00	8.00	7.90
Sodium chloride	26.73	26.73	26.39
Magnesium	2.00	2.00	1.97
	ppm, as-fed		
Iron	5695	5695	5695
Zinc	4015	4015	4015
Manganese	2225	2225	2225
Copper	500	500	500
Iodine	50	50	50
Cobalt	50	50	50
Selenium	——	26	26
	IU/kg, as-fed		
Vitamin A	102272	102272	102272
Vitamin D_3	10227	10227	10227
Vitamin E	23	23	23

[a]Manufactured by Southeastern Minerals, Inc., Bainbridge, GA; served as basal mineral mixture.
[b]Manufactured by Southeastern Minerals, Inc., Bainbridge, GA, by addition of 26 mg/kg Se as sodium selenite to basal mineral mixture.
[c]Created by addition of 1.3% Se yeast (Sel-Plex 2000; Alltech, Inc., Nicholasville, KY) to basal mineral mixture.

Table 6-2. Frequency, daily amount, and total of amount of supplemental Se administered to cows

Source of supplemental Se	Selenium supplementation interval, d	Avg supplementation, mg Se·cow^{-1}·d^{-1}	Total Se supplementation, mg
No Se supplementation	—[6]	—[6]	—[6]
Barium selenate injection[1]	365	1.23	450
Sodium selenite injection[2]	125	0.21	75
Sodium selenite via free-choice minerals[3]	1[5]	1.08	393
Selenized yeast via free-choice minerals[4]	1[5]	2.22	811

[1]Cows received a subcutaneous injection of 9 mL Deposel at initiation of study.
[2]Cows received an injection of 5 mL Mu-Se at initiation of study and re-injection every 4 mo.
[3]Cows had continuous access to free-choice mineral mix containing 26 mg Se/kg as sodium selenite and consumed mineral mix at avg of 41.5 g·cow^{-1}·d^{-1}.
[4]Cows had continuous access to free-choice mineral mix containing 26 mg Se/kg as Se yeast and consumed mineral mix at an avg of 85.5 g·cow^{-1}·d^{-1}.
[5]Access to free-choice minerals containing Se was continuous throughout the study.
[6]Cows receiving no Se supplementation or injectable Se had free-choice access to and consumed the basal free-choice mineral mix (no Se) at an avg of 62.2 g·cow^{-1}·d^{-1}.

Table 6-3. Initial and ending BW of beef cows receiving different sources and forms of Se supplementation[a]

Source of Se supplementation	Initial BW, kg	Ending BW, kg
Control[1] (No Se)	419[b] ± 21	451[bc] ± 21
Barium Selenate[2] (Deposel)	402[b] ± 19	418[b] ± 19
Sodium Selenite[3] (Mu-Se)	419[b] ± 18	453[bc] ± 18
Free Choice Mineral[4] (Selenite)	422[b] ± 19	502[cd] ± 19
Free Choice Mineral[5] (Sel-Plex)	421[b] ± 19	509[d] ± 19

[a]Data represent least squares means and SE, n = 39 for initial and ending BW.
[b,c,d]Means within columns lacking a common superscript differ ($P < 0.05$).
[1]Cows consumed free-choice mineral mix with no Se at an avg of 62.2 g·cow^{-1}·d^{-1} beginning at d 0.
[2]Cows received a subcutaneous injection of 9 mL Deposel at d 0.
[3]Cows received an injection of 5 mL Mu-Se every 4 mo beginning at d 0.
[4]Cows consumed free-choice mineral mix containing 26 mg Se/kg as sodium selenite at an avg of 41.5 g·cow^{-1}·d^{-1} beginning at d 0.
[5]Cows consumed free-choice mineral mix containing 26 mg Se/kg as Se yeast at an avg of 85.5 g·cow^{-1}·d^{-1} beginning at d 0.

Table 6-4. Whole blood Se concentrations of cows receiving different sources and forms of Se supplementation at various d postpartum[a]

	Days postpartum			
	0	30	90	205
Source of Se supplementation	Whole blood Se, µg/L			
Control (No Se)	143[b] ± 15	162[b] ± 15	121[b] ± 15	74[b] ± 15
Barium Selenate[1] (Deposel)	235[e] ± 12	207[cd] ± 12	166[de] ± 12	156[e] ± 12
Sodium Selenite[2] (Mu-Se)	173[b] ± 13	178[bc] ± 12	127[bc] ± 12	89[b] ± 12
Free Choice Mineral [3] (Selenite)	159[b] ± 12	184[bc] ± 13	140[cd] ± 13	155[e] ± 13
Free Choice Mineral [4] (Sel-Plex)	216[e] ± 12	241[d] ± 12	185[e] ± 12	198[d] ± 12

[a]Data represent least squares means and standard errors; n = 41/d; Adequate Se level in whole blood is > 100 µg/L.
[b,c,d,e]Means within columns lacking a common superscript differ ($P < 0.05$).
[1]Cows received a subcutaneous injection of 9 mL Deposel at d 0.
[2]Cows received an injection of 5 mL Mu-Se every 4 mo beginning at d 0.
[3]Cows consumed free-choice mineral mix containing 26 mg/kg Se as sodium selenite at an avg of 41.5 g·cow^{-1}·d^{-1} beginning at d 0.
[4]Dams of these calves consumed free-choice mineral mix containing 26 mg/kg Se as Se yeast at an avg of 85.5 g·cow^{-1}·d^{-1} beginning at d 0.

Table 6-5. Milk Se concentrations of cows receiving different sources and forms of Se supplementation at various d postpartum[a]

	Days postpartum			
	0	30	90	205
Source of Se supplementation	Milk Se, μg/L			
Control (No Se)	$39^{b} \pm 7$	$14^{b} \pm 7$	$6^{b} \pm 7$	$15^{b} \pm 7$
Barium Selenate[1] (Deposel)	$34^{b} \pm 6$	$15^{b} \pm 6$	$15^{b} \pm 6$	$21^{b} \pm 6$
Sodium Selenite[2] (Mu-Se)	$35^{b} \pm 6$	$13^{b} \pm 6$	$6^{b} \pm 6$	$16^{b} \pm 6$
Free Choice Mineral[3] (Selenite)	$39^{b} \pm 7$	$26^{b} \pm 6$	$16^{b} \pm 6$	$15^{b} \pm 7$
Free Choice Mineral[4] (Sel-Plex)	$71^{c} \pm 6$	$27^{b} \pm 6$	$15^{b} \pm 6$	$42^{c} \pm 6$

[a]Data represent least squares means and standard errors; n=41 for each sample d.
[b,c]Means within columns lacking a common superscript differ ($P < 0.05$).
[1]Cows received a subcutaneous injection of 9 mL Deposel at d 0.
[2]Cows received an injection of 5 mL Mu-Se every 4 mo beginning at d 0.
[3]Cows consumed free-choice mineral mix containing 26 mg/kg Se as sodium selenite at an avg of 41.5 g·cow^{-1}·d^{-1} beginning at d 0.
[4]Dams of these calves consumed free-choice mineral mix containing 26 mg/kg Se as Se yeast at an avg of 85.5 g·cow^{-1}·d^{-1} beginning at d 0.

Table 6-6. Plasma Se concentration at d 0 and d 365 of beef cows that received different sources and forms of Se supplementation[a]

	d 0		d 365	
Source of Se Supplementation	Plasma Se, μg/L	SE	Plasma Se, μg/L	SE
Control (No Se)	62^{b}	5	35^{b}	5
Barium Selenate[1] (Deposel)	61^{b}	4	71^{c}	4
Sodium Selenite[2] (Mu-Se)	56^{b}	4	28^{b}	4
Free Choice Mineral[3] (selenite)	52^{b}	4	54^{d}	5
Free Choice Mineral[4] (Sel-Plex)	56^{b}	4	90^{e}	4

[a]Data represent least squares means and standard errors; n = 42 and 41 for d 0 and d 365, respectively; Adequate Se level in plasma is > 70 μg/L.
[b,c,d,e]Means within columns lacking a common superscript differ ($P < 0.05$).
[1]Cows received a subcutaneous injection of 9 mL Deposel at d 0.
[2]Cows received an injection of 5 mL Mu-Se every 4 mo beginning at d 0.
[3]Cows consumed free-choice mineral mix containing 26 mg Se/kg as sodium selenite at an avg of 41.5 g·cow^{-1}·d^{-1} beginning at d 0.
[4]Cows consumed free-choice mineral mix containing 26 mg Se/kg as Se yeast at an avg of 85.5 g·cow^{-1}·d^{-1} beginning at d 0.

Table 6-7. Liver Se concentration (DM basis) at d 0 and d 365 of beef cows that received different sources and forms of Se supplementation[a]

	d 0		d 365	
Source of Se Supplementation	Liver Se, µg/kg	SE	Liver Se, µg/kg	SE
Control (No Se)	973[b]	129	642[b]	129
Barium Selenate[1] (Deposel)	1136[b]	105	1240[c]	105
Sodium Selenite[2] (Mu-Se)	946[b]	105	537[b]	105
Free Choice Mineral[3] (selenite)	1089[b]	105	1046[c]	111
Free Choice Mineral[4] (Sel-Plex)	1011[b]	105	1604[d]	105

[a]Data represent least squares means and standard errors; n = 42 and 41 for d 0 and d 365, respectively; Adequate Se concentration in liver is > 1200 µg/kg.

[b,c,d]Means within columns lacking a common superscript differ ($P < 0.05$).

[1]Cows received a subcutaneous injection of 9 mL Deposel at d 0.

[2]Cows received an injection of 5 mL Mu-Se every 4 mo beginning at d 0.

[3]Cows consumed free-choice mineral mix containing 26 mg Se/kg as sodium selenite at an avg of 41.5 g·cow^{-1}·d^{-1} beginning at d 0.

[4]Cows consumed free-choice mineral mix containing 26 mg Se/kg as Se yeast at an avg of 85.5 g·cow^{-1}·d^{-1} beginning at d 0.

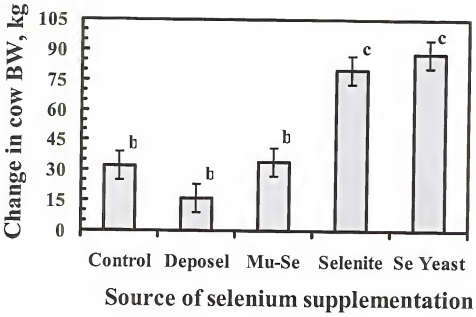

Figure 6-1. Effect of source of selenium supplementation on change in BW in grazing beef cows; Changes in BW lacking a common superscript differ ($P < 0.05$); SEM = 6.92.

CHAPTER 7
TISSUE AND BLOOD SELENIUM CONCENTRATIONS AND PERFORMANCE OF BEEF CALVES FROM DAMS RECEIVING DIFFERENT FORMS OF SELENIUM SUPPLEMENTATION

Introduction

Selenium is required as a coenzyme for glutathione peroxidase, which acts as part of the cellular antioxidant defense system (NRC, 1996), and is associated with the pathogenesis of white muscle disease. White muscle disease, a degeneration of striated, skeletal muscles is the major outward sign of Se deficiency in newborn calves and may develop in utero or shortly after birth (McDowell, 2003). Selenium is also important for adequate immune function. Underdeveloped, weak, dead, or generally unthrifty calves may be born to Se deficient brood cows (Corah and Ives, 1991). Unfortunately, affliction with white muscle disease and poor animal performance due to insufficient Se is not limited to newborn livestock. Calves are susceptible to a delayed WMD, developing after birth, usually from one to four mo of age. Likewise, in Florida and other parts of the southeastern U.S., conditions known as buckling and shoulder lameness are observed in Se deficient feeder calves (McDowell et al., 2002). The costs associated with these conditions and deficiencies may seriously reduce the profit margin of stocker or feedlot operations (Pirelli et al., 2000).

Recent studies indicate that blood Se in newborn calves can be increased through Se supplementation of their dams (Abdelrahman and Kincaid, 1995; Gunter et al., 2003; Valle et al., 2003). Likewise, positive correlations between Se concentration in dam's milk and Se concentration of calf whole blood have been observed in calves up to 70 d of

age (Pehrson et al., 1999). Evidence also exists that milk Se can be increased by level and duration of Se supplementation in lactating cows (Conrad and Moxon, 1979). Given these findings it seems logical that calf blood Se can be increased from birth to weaning by supplementing the cow herd with Se. We hypothesized that Se status of calves could be improved through Se supplementation of their dams and that an organic source of Se may be more efficient at maintaining adequate blood and tissue Se. Thus, this experiment was designed to evaluate and compare effects on blood and tissue Se concentrations of calves born to and suckling dams that received different sources and physical forms of supplemental Se. It should be noted that each supplementation method evaluated in this study is readily available to livestock producers.

Materials and Methods

All animal procedures were conducted within the guidelines of and approved by the University of Florida Institutional Animal Care and Use Committee. Animals were housed at and all sampling took place at the University of Florida Boston Farm-Santa Fe Beef Unit located in Northern Alachua County, Florida. On August 6, 2002, 43 Angus cows, aged 2-3 yr, (mean age = 2.67 yr) were palpated to diagnose pregnancy and estimate d in gestation. All cows were determined pregnant and gestation estimates ranged from 115 to 130 d. Each animal received a chemically altered modified live four-way viral + vibriosis and leptospirosis vaccination (Cattlemaster 4+VL-5; Pfizer Animal Health, Exton, PA) and fly control (Permectrin 10% EC pour-on; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) according to manufacturers' directions. Cows were weighed (average BW = 417 ± 46 kg), stratified by age, and assigned to one of five treatment groups for a 365 d study. The five treatments were 1) no Se supplementation, (control), 2) one subcutaneous injection of 9 mL (50 mg Se·mL^{-1}) barium selenate

(Deposel Multidose; Novartis New Zealand, Ltd., Auckland, NZ) at the initiation of the experiment, 3) three subcutaneous injections of 5 mL (5 mg Se·mL^{-1} + 68 IU vitamin E as dl-α-tocopheryl·mL^{-1}) of sodium selenite (Mu-Se; Schering-Plough Animal Health, Union, NJ) at the initiation of the experiment and every four mo thereafter, 4) free-choice access to a mineral mixture containing 26 mg Se/kg as sodium selenite (Southeastern Minerals, Inc., Bainbridge, GA), or 5) free-choice access to a mineral mixture containing 26 mg Se/kg as Se yeast (Sel-Plex; Alltech, Inc, Nicholasville, KY). All cows grazed bahiagrass (*Paspalum notatum*) pastures at a stocking rate of 1.7 cows/ha and were supplemented with bahiagrass (*Paspalum notatum*) hay, molasses-based liquid supplement ad libitum, and whole cottonseed and pelleted citrus pulp at rates of 0.68 kg·cow^{-1}·d^{-1} and 1.81 kg·cow^{-1}·d^{-1}, respectively, from November 2002 through March 2003.

Treatment groups receiving no Se or injectable Se were housed together and had access to a free-choice mineral mixture containing no Se (Table 7-1). Cows receiving Se via free-choice mineral mixtures were housed in separate groups and had access to the same mineral mixture with added Se as sodium selenite or Se yeast for treatments 4 and 5, respectively (Table 7-1). All free-choice mineral mixtures were offered in wooden mineral feeders and protected from rain and at a height to prevent calves from consuming the mineral mixtures.

Forty-two calves (25 male, 17 female) were born over a 24 d span between December 31, 2002 and January 23, 2003. Average age of calf did not differ among treatment groups ($P = 0.40$) and calves had an average birth date of January 10, 2003. Blood samples from calves were collected via jugular venipuncture into 10-mL

heparinized tubes (Vacutainer; Becton-Dickinson, Franklin Lakes, NJ) immediately after birth (d 0) and before nursing. If technicians were unable to determine whether or not the calf had nursed, then no sample was taken. This was done so that calf blood Se concentration at birth could be attributed to maternal transfer of Se rather than from Se obtained via colostrum. Additional blood samples were collected in the same manner from all calves on d 30, d 90, and d 205. On these dates, calves were an average age of 33, 88, and 208 d, respectively. Twenty-two of the male calves were surgically castrated immediately after birth and the testes were then frozen (0°C) until analysis. Liver biopsies were performed on all calves at d 205, which coincided with weaning, and liver samples were placed on ice and then frozen (0°C) until analysis. Body weight was recorded for all calves at d 0 and at weaning (d 205). Body weight at weaning and actual age of calf in d was used to calculate ADG. Additionally, samples of cow blood and milk were collected immediately following parturition and on the same subsequent dates as the calves. Samples from cows were collected and stored in the same manner as those taken from the calves.

Pastures were sampled in October 2002 (before calving season), March 2003 (after calving season), and August 2003 (at weaning). Likewise, hay, molasses-based liquid supplement, whole cottonseed, and pelleted citrus pulp were sampled during winter supplementation. Whole blood, liver, testes, and all feedstuffs were analyzed for Se concentration using a fluorometric method described by Whetter and Ullrey (1978). To help ensure reliability of the analytical method, a certified standard (National Bureau of Standards Bovine Liver SRM-1577a; U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) was frequently analyzed.

Effects of treatment on birth weight, weaning weight, ADG, liver Se, and testes Se were analyzed using PROC GLM (SAS for Windows 8e; SAS Inst., Inc., Cary, NC) in a completely randomized design. Pre-planned contrast statements were used to compare means as described by Littell et al. (1998; 2000). PROC MIXED (SAS for Windows 8e; SAS Inst., Inc., Cary, NC) was used to analyze effects of treatment, d, and the interaction of treatment × d on calf whole blood as repeated measures with a spatial power covariance structure with respect to d and subplot of animal nested within treatment. Pre-planned contrast statements were written to determine differences in means for different sampling d. PROC CORR was used to determine correlations of calf whole blood Se and calf liver Se to cow whole blood and milk Se.

Results

Selenium concentration of pasture and hay for all groups averaged 0.071 and 0.045 mg/kg DM basis, respectively. Crude protein was 12.57% for hay and 12.79% ± 1.76 for standing forage. Forage CP did not differ ($P = 0.92$) during the study. Whole cottonseed, pelleted citrus pulp, and liquid supplement analyzed 0.002, 0.002, and 0.744 mg Se/kg, (as-fed) respectively. Free-choice mineral consumption was monitored and recorded. Mineral consumptions and amount of Se via injection are summarized in Table 7-2. All free-choice mineral mixtures had been formulated for an expected intake of 85-113 $g \cdot cow^{-1} \cdot d^{-1}$. However, only the cows on the Se yeast treatment had mineral consumption in this range (Table 7-2). Treatment groups receiving free-choice minerals with no Se consumed 62.19 $g \cdot cow^{-1} \cdot d^{-1}$ and cows receiving free-choice minerals with sodium selenite consumed 41.5 $g \cdot cow^{-1} \cdot d^{-1}$.

There was no effect of form Se supplementation to cows on calf birth weight ($P = 0.83$) or weaning weight ($P = 0.75$) at 205 d (Table 7-3). Conversely, calf ADG was

affected by Se supplementation of dams ($P = 0.04$) and there were differences ($P < 0.05$) in ADG among treatment groups. Both free-choice treatments produced higher ($P < 0.05$) calf ADG than did the injectable barium selenate treatment and calf ADG from both free-choice treatments tended to be higher ($P = 0.10$) and ($P = 0.07$), respectively, than ADG in calves whose dams received Se supplementation as injectable sodium selenite (Table 7-3).

Treatment and treatment × d affected ($P < 0.001$) Se concentrations in calf whole blood (Table 7-4). Differences ($P < 0.05$) existed among treatment groups on all sampling d. At d 0, calves from Se yeast treated cows had higher ($P < 0.05$) whole blood Se than did those on control, injectable selenate, or free-choice selenite treatments and tended ($P < 0.10$) to have higher blood Se than calves from injectable selenite treated cows.

Whole blood Se concentrations were higher ($P < 0.05$) in calves from the Se yeast treatment than those from all other treatment groups and no other differences or tendencies ($P = 0.30$) were observed at d 30. Again at d 90, the Se yeast treatment produced blood Se concentrations higher ($P < 0.05$) than all other treatments. The two injectable treatments were similar to control ($P = 0.64$) and the free-choice selenite treatment was higher ($P < 0.05$) than injectable selenite. Furthermore, the free-choice selenite treatment tended ($P = 0.15$) to be higher than control and injectable selenate treatments.

At d 205 (weaning), the two injectable treatments produced calf whole blood Se similar ($P = 0.56$) to controls. The free-choice selenite treatment produced calf whole blood Se higher ($P < 0.05$) than either injectable product and tended ($P = 0.08$) to be

higher than control. Again at d 205, as was observed at d 30 and d 90, calves on the Se yeast treatment had higher ($P < 0.05$) blood Se than calves on all other treatments. The two injectable products produced blood Se that was similar ($P = 0.33$) on all sampling d. A linear decrease ($P < 0.001$) in calf whole blood Se was observed across all sampling d.

Selenium concentrations in testes taken from calves at birth did not differ among treatment groups (Table 7-5). Although, testicular Se from the injectable selenate group tended to be higher ($P = 0.07$) than testicular Se from the free-choice selenite treatment. Liver Se (DM basis) taken from calves at 205 d was affected ($P < 0.001$) by form of Se supplementation administered to their dams (Table 7-5). Calves whose dams received Se as Se yeast had higher ($P < 0.05$) liver Se than calves from all other treatment groups. Likewise, calves whose dams received sodium selenite via free-choice minerals had higher ($P < 0.05$) liver Se than calves on control and both injectable treatments. Calf liver Se from control did not differ ($P = 0.48$) from either injectable treatment, and calf liver Se from the two injectable Se sources were similar ($P = 0.90$).

Selenium concentrations from whole blood collected from cows and their calves on d 30, 90, and 210 were used to correlate calf blood Se to cow blood Se. Correlations between Se concentration in cow whole blood and calf liver were calculated from samples collected at d 205. Figure 7-1 exhibits the positive correlation ($r = 0.64$; $P < 0.001$) between Se concentration in cows' whole blood and Se concentration in the whole blood of their calves across all sampling d. When correlations between cows' and calves' whole blood Se were calculated on individual sampling d, positive ($P < 0.001$) correlations were also observed ($r = 0.65$, 0.68, and 0.52) in samples taken at d 30, 90, and 205, respectively. Figure 7-2 shows the positive ($P < 0.001$) correlation ($r = 0.57$)

between Se in cows' whole blood and liver Se of their calves at d 205. The positive ($P <$ 0.003) correlation (r = 0.049) between Se in cows' milk and Se in whole blood of their calves is illustrated in Figure 7-3.

Discussion

Birth weights of the calves (average 35 kg) were similar to those reported in other studies of this type (Ammerman et al., 1980; Gunter et al., 2003; Valle et al., 2003). Likewise, these authors reported no significant differences in calf birth weight among treatment groups. Unadjusted weaning weight of calves on our study did not differ among treatment groups, though calves whose dams received Se yeast were numerically heavier than those from all other treatments. Gunter et al. (2003) compared effects of Se supplementation via sodium selenite or Se yeast to beef cows on calf performance and found no differences in total weight gain and ADG in calves. However, Spears et al. (1986) found significant differences in summer weight gain and adjusted weaning weight in crossbred beef calves supplemented with injectable Se and vitamin E. Hidiroglou and Jenkins (1975) also reported weight gain in calves administered Se with vitamin E. Additionally, Castellan et al. (1999) observed increased ADG in Angus × Hereford calves that were supplemented with injectable sodium selenite at intervals before weaning. Differences observed in calf ADG in this study somewhat follow the pattern of differences in calf blood Se at weaning. At weaning, the control and both injectable groups had similar blood Se and similar ADG. In our experiment, calves from both free-choice treatments had higher blood Se than calves whose dams received injectable Se and ADG was higher in the free-choice groups than in the injectable selenate group. Wichtel et al. (1996) reported an increase of 20% in ADG of Se supplemented Friesian heifers vs unsupplemented controls. Those authors also indicated the possibility of altered growth

rate due to a Se deficiency and its effects on thyroid hormones. In the present study, increases in ADG of 8, 11, and 19% were observed in calves from cows supplemented with Se via free-choice minerals over those calves from control, injectable selenite, and injectable barium selenate treated cows, respectively. These increases in ADG could be somewhat due to calves' enhanced immune function and decreased affliction with subclinical illnesses.

The critical whole blood Se concentration for cattle is controversial; however > 100 μg Se/L is defined as adequate by several authors (Hansen et al., 1991; Gerloff, 1992; Dargatz and Ross, 1996). Those authors also define a blood Se of < 75 μg Se/L as marginal or subclinical. Blood Se < 50 μg Se/L is accepted as deficient by Pehrson et al. (1999) and Gerloff (1992). Furthermore, Dargatz and Ross designate that concentration as severely deficient. We will use the aforementioned concentrations and designations to discuss our findings as they relate to calf whole blood Se.

At birth, all calves on this study had adequate blood Se concentrations ranging from 104 μg Se/L (control) to 200 μg Se/L (Se yeast). Both free-choice and the injectable barium selenate treated cows had calves with higher blood Se than did controls. Injectable selenite also produced numerically higher concentrations than control. These results concur with the findings of Gunter et al. (2003) where higher blood Se was found in calves whose dams were supplemented with Se yeast (203 μg Se/L) vs sodium selenite (134 μg Se/L) in their free-choice minerals. In the same study, both forms of Se supplementation produced higher blood Se than did no Se supplementation.

By d 30, whole blood Se was still lowest at 86 μg Se/L for the control animals and whole blood Se remained highest for the Se yeast treated animals at 166 μg Se/L.

Both the injectable and the free-choice selenite treatments produced whole blood Se similar to control. This agrees with Valle et al. (2003) who measured calf whole blood Se at 60 d. Such decreases are likely due to calves relying solely on a milk diet and depleting blood Se and liver reserves which are reported to be bolstered by Se supplementation to pregnant cows (Koller et al., 1984; Abdelrahman and Kincaid, 1995).

Calf blood Se had dropped below marginally adequate levels in the control and both injectable treatment groups at d 30. Calves from the free-choice selenite group had blood Se in the marginally adequate range and the Se yeast treatment had blood Se values nearly two-fold higher than each of the other treatments. Gunter et al. (2003) also reported whole blood Se from Se yeast treatments to be at least two-fold higher than sodium selenite or unsupplemented calves at about 110 d of age. Results from Valle et al. (2003) followed a similar pattern, as plasma Se in calves from Se yeast supplemented dams was three to four higher than calves whose dams had no Se supplementation or injectable Se supplementation. At weaning (d 205), calves from control, injectable selenate, and injectable selenite treatment groups had blood Se of 42, 34, and 36 μg Se/L, respectively, all of which were considerably below the deficient threshold. Calves from dams that received free-choice selenite were classified as marginally deficient and the Se yeast treatment produced blood Se well over the adequate level and more than five-fold higher than either injectable product at weaning. Blood Se of calves from dams receiving injectable Se or the free-choice selenite were similar during the majority of this study and decreased with calf age. Days 90 and 205 of this study were in April and early August. At these times, in Florida and most of the United States, grasses are growing rapidly and contain higher concentrations of unsaturated fatty acids. It has been postulated by

Pehrson et al. (1999) that during such times cows would have greater oxidative stresses due to diet. If so, inorganic Se supplements may not be adequate to maintain Se concentrations in cow blood and milk. Results from this study, as well as results from Gunter et al. (2003), Valle et al. (2003), and Pehrson et al. (1999) all demonstrate decreases in blood Se of calves, whose dams received Se via inorganic sources at times of lush forage growth. These results support the aforementioned postulation, but investigation of antioxidants such as blood α-tocopherol levels are likely needed for definitive support.

In the present study, testes of male calves taken at birth were used as an alternative to liver in an effort to evaluate maternal transfer of Se to calf tissues. Selenium concentrations in calf testes taken at birth were not statistically different but did follow a similar numeric pattern to the liver Se concentrations of beef cows receiving either Se yeast or injectable Se (Valle, 2001). In our study, testis Se concentration in calves whose dams received Se yeast or injectable barium selenate was approx 29% higher than controls. Researchers in India demonstrated greater oxidative stress in the testes of mice when less Se was included in their diets (Kaur and Bansal, 2004), but data relating to Se concentrations in calf testes is limited.

Liver samples, on a dry basis, taken at weaning had Se concentrations (μg/kg DM) that were on average 7.89 times greater than whole blood Se (μg/L) collected on the same d. Likewise, liver and whole blood Se followed a very similar pattern to whole blood, as calves from dams receiving Se yeast were higher than all other calves in whole blood and liver Se. Control and both injectable products were similar for most all measurements and free-choice selenite produced liver and whole blood Se higher than

both injectable products. These data agree with Abdelrahman and Kincaid (1995) where liver Se at d 0 and d 42 was more than two-fold higher for Se supplemented than for unsupplemented Holstein calves. These authors continue by stating that their data help emphasize the need for Se supplementation to newborn calves in Se deficient areas. Our findings help support such a need. Liver Se of 2200 μg/kg in calves is required for normal growth and health (Van Saun et al., 1989). Though liver samples were not taken at birth, Se concentrations in liver at weaning failed to even approach this threshold and in control animals liver Se concentrations were less than 20% of this recommended concentration. Also, only calves from dams that received Se yeast had liver Se concentrations at weaning that would be considered to be in the normal range (Stowe and Herdt, 1992). Valle (2001) reported liver Se that averaged 730 μg/kg in yearling cattle that had been supplemented with sodium selenite and 930 μg/kg when Se was supplemented via Se yeast. Our current findings concur with these concentrations as well as concentrations in dairy cows reported by Knowles et al. (1999). Regardless of liver or whole blood Se status, no signs of WMD or buckling were observed in the calves on this study, even during times of stress (e.g., liver biopsy or weaning).

The correlation between cow whole blood and calf whole blood in our study was positive ($r = 0.64$) and suggests that Se supplementation of the cow herd is an effective means of supplementing Se to nursing calves. The correlation between calf liver Se at weaning and cow whole blood Se concentration ($r = 0.57$) further supports this suggestion. Pehrson et al. (1999) reported positive correlations of $r = 0$ .64, 0.68, and 0.59 between calf blood Se, calf plasma Se, and glutathione peroxidase activity in calf blood to cow milk Se concentration. These correlation values are somewhat higher than

from our study, where a correlation value of r = 0.49 was observed between the concentration of Se in cows' milk and the Se in the whole blood of their calves. This value may have been more positive if Se concentrations from Se rich colostrum were not included in the correlation.

Implications

Supplementation of selenium to the cow herd with injectable selenium or with sodium selenite in free-choice minerals was not shown to be an effective method to attain adequate blood selenium in pre-weaned calves. Therefore, calves whose dams are receiving either no selenium or selenium supplementation via these methods may be susceptible to disease and suppressed growth due to selenium deficiency. Calves suckling dams that were supplemented with organic selenium as selenium yeast, however, have been found to have adequate blood selenium from birth to weaning. These calves should be at little or no risk of disease due solely to selenium deficiency.

Summary

The objective of this experiment was to evaluate and compare effects of different sources and physical forms of supplemental Se fed to cows on blood and tissue Se concentrations of their calves. Forty-three Angus cows (115-130 d gestation) were randomly assigned to five groups and received either no Se supplementation (control), one 9 mL barium selenate s.c injection, 5 mL sodium selenite via s.c. injection, every four mo, or sodium selenite or Se yeast at 26 mg Se/kg in free-choice mineral mixtures. Cows grazed bahiagrass (*Paspalum notatum*) pastures, received routine dietary supplementation and calved mostly in early January 2003. No differences in calf weight at birth or weaning were observed between treatment groups. Average daily gain was higher ($P < 0.05$) for calves from dams that received Se via free-choice minerals. Calf

whole blood Se was determined at birth, 30, 90 and 205 d of age. Testicles were collected at birth from males and a liver biopsy was performed on all calves at d 205 for liver Se determination. At birth, calf blood Se was in the adequate range (>100 μg Se/L) for all treatments. At d 30 and d 90, control, both injectable products, and free-choice sodium selenite treatments produced calf blood Se in or near the adequate range, and calves from Se yeast treated cows maintained blood Se well above the adequate range at 166 and 182 μg Se/L at d 30 and 90, respectively. At 205 d, whole blood Se concentrations for control and both injectables were in the deficient range (< 50 μg Se/L), calves from the free-choice sodium selenite treatment were marginally deficient (< 75 μg Se/L) and the Se yeast group had blood Se well above adequate at 188 μg Se/L. Selenium in calf testes ranged from 162 to 210 μg Se/kg DM and did not differ among treatments. Liver Se taken at weaning ranged from 297 to 1321 μg Se/kg DM and calves from the two free-choice treatments had Se concentrations higher ($P < 0.05$) than calves from both injectable treatments and the controls. Positive correlations ($r = 0.57$ and 0.64; $P < 0.01$) between calf liver Se and calf whole blood Se, to cow whole blood Se were observed. Correlation between concentration of Se in calf whole blood and in cow milk was also positive ($r = 0.49$; $P < 0.002$). Supplementation of Sel-Plex to cows produced adequate calf blood Se throughout this experiment, whereas control and the other treatments produced calf blood Se concentrations that were mostly in the marginal or deficient range. Supplementation of Se as Se yeast to the cow herd is an effective means of maintaining adequate blood Se in pre-weaned calves.

Table 7-1. Composition of mineral mixtures offered free-choice to brood cows

Component	Free-choice mineral mixtures		
	No Se[a]	Sodium selenite[b]	Selenized yeast[c]
	%, as-fed		
Calcium	18.73	18.73	18.49
Phosphorus	8.00	8.00	7.90
Sodium chloride	26.73	26.73	26.39
Magnesium	2.00	2.00	1.97
	ppm, as-fed		
Iron	5695	5695	5695
Zinc	4015	4015	4015
Manganese	2225	2225	2225
Copper	500	500	500
Iodine	50	50	50
Cobalt	50	50	50
Selenium	—	26	26
	IU/kg, as-fed		
Vitamin A	102272	102272	102272
Vitamin D_3	10227	10227	10227
Vitamin E	23	23	23

[a]Manufactured by Southeastern Minerals, Inc., Bainbridge, GA; served as basal mineral mixture.
[b]Manufactured by Southeastern Minerals, Inc., Bainbridge, GA, by addition of 26 mg/kg Se as sodium selenite to basal mineral mixture.
[c]Created by addition of 1.3% Se yeast (Sel-Plex 2000; Alltech, Inc., Nicholasville, KY) to basal mineral mixture.

Table 7-2. Frequency, daily amount, and total of amount of supplemental Se administered to cows

Source of supplemental Se	Selenium supplementation interval, d	Avg supplementation, mg Se·cow^{-1}·d^{-1}	Total Se supplementation, mg
No Se supplementation	—[6]	—[6]	—[6]
Barium selenate injection[1]	365	1.23	450
Sodium selenite injection[2]	125	0.21	75
Sodium selenite via free-choice minerals[3]	1[5]	1.08	393
Selenized yeast via free-choice minerals[4]	1[5]	2.22	811

[1]Cows received a subcutaneous injection of 9 mL Deposel at initiation of study.
[2]Cows received an injection of 5 mL Mu-Se at initiation of study and re-injection every 4 mo.
[3]Cows had continuous access to free-choice mineral mix containing 26 mg Se/kg as sodium selenite and consumed mineral mix at avg of 41.5 g·cow^{-1}·d^{-1}.
[4]Cows had continuous access to free-choice mineral mix containing 26 mg Se/kg as Se yeast and consumed mineral mix at an avg of 85.5 g·cow^{-1}·d^{-1}.
[5]Access to free-choice minerals containing Se was continuous throughout the study.
[6]Cows receiving no Se supplementation or injectable Se had free-choice access to and consumed the basal free-choice mineral mix (no Se) at an avg of 62.2 g·cow^{-1}·d^{-1}.

Table 7-3. Birth weights, weaning weights, and ADG of calves from dams receiving different sources and forms of Se supplementation[a]

Source of Se supplementation	Birth wt, kg	Weaning wt, kg	ADG, kg
Control (No Se)	$35.3^{b} \pm 3.1$	$213.5^{b} \pm 17.8$	$0.85^{bc} \pm .04$
Barium Selenate[1] (Deposel)	$34.0^{b} \pm 2.5$	$194.9^{b} \pm 15.5$	$0.78^{b} \pm .04$
Sodium Selenite[2] (Mu-Se)	$34.3^{b} \pm 2.5$	$209.2^{b} \pm 14.6$	$0.83^{bc} \pm .03$
Free Choice Mineral[3] (selenite)	$37.5^{b} \pm 2.5$	$195.5^{b} \pm 15.5$	$0.92^{c} \pm .04$
Free Choice Mineral[4] (Sel-Plex)	$33.6^{b} \pm 2.5$	$219.3^{b} \pm 15.5$	$0.93^{c} \pm .04$

[a]Data represent least squares means, n = 42, 39, 39 for birth wt, 205 d weaning wt, and ADG, respectively.

[b,c,d]Means within columns lacking a common superscript differ ($P < 0.05$).

[1]Dams received a subcutaneous injection of 9 mL Deposel at an avg of 125 d gestation.

[2]Dams received an injection of 5 mL Mu-Se every 4 mo beginning at an avg of 125 d gestation.

[3]Dams of these calves consumed free-choice mineral mix containing 26 mg/kg Se as sodium selenite at an avg of 41.5 $g \cdot cow^{-1} \cdot d^{-1}$ beginning at an avg of 125 d gestation.

[4]Dams of these calves consumed free-choice mineral mix containing 26 mg/kg Se as Se yeast at an avg of 85.5 $g \cdot cow^{-1} \cdot d^{-1}$ beginning at an avg of 125 d gestation.

Table 7-4. Calf whole blood Se at various ages from dams receiving different sources and forms of Se supplementation[a]

	Age of calf, d			
	0	30	90	205
Source of Se supplementation	Whole blood Se, μg/L			
Control (No Se)	$104^{b} \pm 24$	$86^{b} \pm 10$	$65^{bc} \pm 10$	$42^{bc} \pm 10$
Barium Selenate[1] (Deposel)	$160^{c} \pm 12$	$100^{b} \pm 8$	$71^{bc} \pm 8$	$34^{b} \pm 8$
Sodium Selenite[2] (Mu-Se)	$155^{bcd} \pm 24$	$98^{b} \pm 8$	$59^{b} \pm 8$	$36^{b} \pm 8$
Free Choice Mineral[3] (selenite)	$156^{c} \pm 11$	$99^{b} \pm 9$	$88^{c} \pm 9$	$67^{c} \pm 10$
Free Choice Mineral[4] (Sel-Plex)	$200^{d} \pm 14$	$166^{c} \pm 9$	$182^{d} \pm 9$	$188^{d} \pm 9$

[a]Data represent least squares means, n = 14, 39, 39, and 39 for d 0, 30, 90, and 205, respectively. At d 0 samples were collected only from calves which had not yet nursed.

[b,c,d]Means within columns lacking a common superscript differ ($P < 0.05$).

[1]Dams of these calves received a subcutaneous injection of 9 mL Deposel at an avg of 125 d gestation.

[2]Dams of these calves received an injection of 5 mL Mu-Se every 4 mo beginning at an avg of 125 d gestation.

[3]Dams of these calves consumed free-choice mineral mix containing 26 mg/kg Se as sodium selenite at an avg of 41.5 $g \cdot cow^{-1} \cdot d^{-1}$ beginning at an avg of 125 d gestation.

[4]Dams of these calves consumed free-choice mineral mix containing 26 mg/kg Se as Se yeast at an avg of 85.5 $g \cdot cow^{-1} \cdot d^{-1}$ beginning at an avg of 125 d gestation.

Table 7-5. Testis and liver Se concentrations (μg/kg, dry basis) of beef calves suckling dams that received different sources of Se supplementation[a]

Source of Se Supplementation	Testes Se, μg/kg	SE	Liver Se, μg/kg	SE
Control (No Se)	162[b]	21	372[b]	82
Barium Selenate[1] (Deposel)	210[b]	17	297[b]	65
Sodium Selenite[2] (Mu-Se)	177[b]	19	308[b]	61
Free Choice Mineral[3] (selenite)	169[b]	14	600[c]	69
Free Choice Mineral[4] (Sel-Plex)	207[b]	21	1321[d]	69

[a]Testes taken at birth; Liver biopsies at 205 d of age; data represent least squares means, n = 22 and 36, respectively.

[b,c,d]Means within columns lacking a common superscript differ ($P < 0.05$).

[1]Dams of these calves received a subcutaneous injection of 9 mL Deposel at an avg of 125 d gestation.

[2]Dams of these calves received an injection of 5 mL Mu-Se every 4 mo beginning at an avg of 125 d gestation.

[3]Dams of these calves consumed free-choice mineral mix containing 26 mg/kg Se as sodium selenite at an avg of 41.5 $g \cdot cow^{-1} \cdot d^{-1}$ beginning at an avg of 125 d gestation.

[4]Dams of these calves consumed free-choice mineral mix containing 26 mg/kg Se as Se yeast at an avg of 85.5 $g \cdot cow^{-1} \cdot d^{-1}$ beginning at an avg of 125 d gestation.

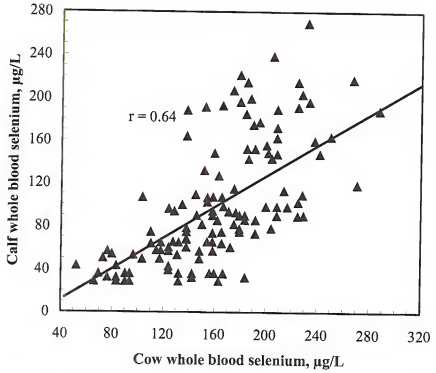

Figure 7-1. Correlation between the concentration of Se in cows' whole blood and the concentration of Se in the whole blood of their calves.

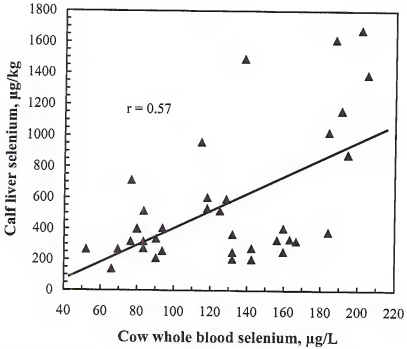

Figure 7-2. Correlation between the concentration of Se in cows' whole blood and the concentration of Se in the liver of their calves.

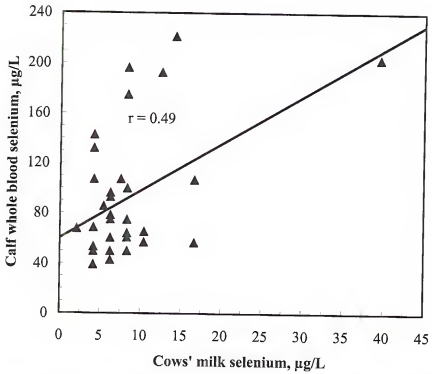

Figure 7-3. Correlation between the concentration of Se in cows' milk and the concentration of Se in the whole blood of their calves.

CHAPTER 8
SUMMARY AND CONCLUSIONS

Selenium has had a long and storied history in animal nutrition and has played the role of toxic element, essential nutrient, carcinogen, and contributor in cancer prevention. However, it seems that selenium's greatest legacy is one of a toxic agent to livestock. With its many implications as a toxic element, the use of Se as a supplement to livestock, garners much caution from feed manufacturers, animal scientists, and nutritionists. The current estimate of the maximum tolerable level for dietary Se in domestic animals is 2 mg/kg. This estimate does not consider differences in metabolism of Se by different species and makes no differentiation in the maximum tolerable level for the different chemical forms of Se, such as Se yeast or sodium selenite. Furthermore, some evidence exists to suggest that the maximum tolerable dietary concentration of Se for livestock is grossly underestimated and to discredit the notion that the range between optimal and toxic levels of Se is narrow. Likewise, studies in cattle and swine have shown a marked difference in the efficacy of organic vs inorganic Se to increase blood, milk, and tissue Se concentrations.

At the University of Florida, two types of experiments were completed. With sheep, studies of tolerance in relation to maximum tolerance levels for ewes and wethers were undertaken. Experiments using sheep were conducted to gather further data on 1) the amount of dietary inorganic Se that can be tolerated by ewes during lamb production, 2) the effects of high levels of dietary Se fed to ewes on their lambs, and 3) the amount of

organic or inorganic Se that can be tolerated by mature wethers. With a cow-calf herd, methods and sources of Se supplementation were evaluated.

A 72-wk study was conducted to determine the maximum tolerable level of selenite Se for ewes during lamb production. The criteria for determining Se tolerance included clinical signs, Se in blood, wool, and tissues, histopathological evaluation, and the activity levels of enzymes related to Se toxicosis. Forty-one range-type ewes were fed Se as sodium selenite which was added to a corn-soybean meal basal diet at levels of 0.2 (control), 4, 8, 12, 16, and 20 mg/kg. Serum Se and ewe body weight (BW) were measured at 4-wk intervals, whole blood Se and wool Se were measured every 12 wk, and samples of brain, diaphragm, heart, hoof, kidney, liver, and loin muscle were collected at the termination of the experiment. Ewe BW was unaffected by dietary Se level ($P = 0.69$). Ewe serum Se, whole blood Se, and wool Se increased linearly as dietary Se increased ($P < 0.05$). Brain, diaphragm, heart, and psoas major muscle Se increased linearly as Se in the diet increased, liver Se responded quadratically, and hoof and kidney Se responded cubically to treatment ($P < 0.05$). In general, serum, whole blood, and tissue Se concentrations from ewes receiving 12, 16, or 20 mg/kg dietary Se were higher ($P < 0.05$) than from controls and ewes receiving less dietary Se. Though serum, whole blood, and wool Se concentrations were elevated in ewes receiving increased dietary Se, at no time did serum, whole blood, or wool Se concentrations reach levels previously reported as toxic and a pattern of clinical signs of Se toxicosis was not observed. Histopathological microscopic evaluation of liver, kidney, diaphragm, heart, and psoas major muscle did not reveal evidence of Se toxicosis in ewes on any dietary Se treatment. Levels of albumin and the activity of Alk phos, ALT, AST, CK, and GGT

were in their respective normal ranges at the end of the study. This further indicates no tissue damage occurred due to Se toxicosis.

Most of the ewes receiving different levels of dietary Se lambed twice during the study. The effects of the six levels of dietary selenium on ewes' milk and the Se status of their lambs prior to weaning were also compared and evaluated. Colostrum Se ranged from 257 to 3542 μg/L and increased linearly as dietary Se increased ($P < 0.001$) in both years. Ewe milk Se ranged from 75 to 2228 μg/L and also increased linearly as dietary Se increased ($P < 0.01$). In general, ewes receiving ≥ 12 mg/kg Se produced higher milk Se than controls. Blood samples were collected from lambs before nursing and at 3, 28, and 56 d of age to evaluate plasma Se concentrations. At birth, lamb plasma Se ranged from 74 to 775 μg/L and was affected ($P < 0.001$) by the Se concentration of the ewe diets, which indicates placental transfer of Se. Lambs from ewes receiving dietary Se at 20 mg/kg had higher ($P < 0.05$) plasma Se than controls at birth and 3, 28, and 56 d of age in both yr. Selenium concentration in testes collected at 70 d of age was also affected by Se content of ewe diets. In yr one, lambs whose dams received 16 or 20 mg/kg Se had higher ($P < 0.05$) testicular Se than controls, but no differences in testicular Se were observed in yr two. No signs of Se toxicosis were observed in lambs regardless of dietary Se concentration of the ewes' diet.

A 60-wk experiment was conducted to determine maximum tolerable of Se by feeding Se as sodium selenite or Se yeast at high dietary levels to wether sheep. Criteria use to determine maximum tolerable levels were the same as those evaluated in the ewe experiment. Twenty-eight crossbred wethers received 0.2, 20, 30, or 40 mg/kg dietary Se (as-fed) from sodium selenite or Se yeast added to a corn-soybean meal basal diet.

Wethers were weighed at 8-wk intervals, serum Se, whole blood Se, and wool Se were measured every 12 wk, and samples of brain, diaphragm, heart, hoof, kidney, liver, loin muscle, and serum samples for evaluation of albumin and enzyme activities were collected at the termination of the experiment. Wether BW was affected by dietary Se level ($P < 0.05$), source of dietary Se ($P < 0.05$), and time ($P < 0.05$), and avg BW decreased linearly ($P < 0.10$) as dietary Se level increased though most wethers gained BW. Serum Se, whole blood Se, and wool Se concentrations were affected ($P < 0.05$) by dietary level of Se and source of Se. Serum Se and whole blood Se ranged from 110 to 3922 μg/L and 392 to 6259 μg/L, respectively, and increased in a quadratic fashion as dietary Se level increased ($P < 0.05$) and wool Se ranged from 1.19 to 39.09 mg/kg and responded linearly ($P < 0.05$) to increased dietary Se. Serum Se, whole blood Se, and wool Se concentrations from wethers receiving organic Se were higher ($P < 0.01$) than those from wethers receiving inorganic Se. Selenium concentrations in brain, diaphragm, heart, hoof, kidney, liver, and loin muscle were affected ($P < 0.05$) by dietary Se level, with higher Se concentrations generally observed in tissues from wethers receiving organic Se. Though Se concentrations in serum, blood, wool, and major organs at most times exceeded concentrations previously reported in livestock suffering from Se toxicosis, a pattern of clinical signs of Se toxicosis was not observed in this experiment. Histopathological, microscopic evaluation of liver, kidney, diaphragm, heart, and loin muscle did not reveal definitive evidence of Se toxicosis in wethers on any dietary Se treatment, and albumin and enzyme activity levels were in their normal respective ranges.

An Angus cow-calf herd was utilized to evaluate and compare effects of using different forms of dietary or parenteral Se on BW change and blood, milk, and liver Se

concentrations of beef cows. In a 365 d study, 43 gestating Angus cows were randomly assigned to five groups and received either no Se supplementation (control), one 5-mL sodium selenite injection s.c. every four mo, one 9-mL barium selenate injection s.c. at the initiation of the study, or free-choice mineral mixtures containing 26 mg/kg Se as sodium selenite or Se yeast. Body weight, plasma Se, and liver Se were measured at d 0 and d 365. Whole blood and milk samples were taken at calving and 30, 90, and 205 d postpartum. Cows receiving Se in free-choice minerals were heavier and had a greater increase in BW at d 365 ($P < 0.05$) than cows receiving all other treatments. Plasma Se and liver Se concentrations were not initially different. At d 365, plasma Se in cows receiving Se yeast was higher ($P < 0.05$) at 90 μg/L than from all other treatments. Injectable selenite was intermediate and produced higher plasma Se than control and both forms of selenite. Liver Se at d 365 was adequate (> 1200 μg/kg) and higher ($P < 0.05$) in Se yeast treated cows than all others. Cows receiving injectable selenate also had adequate liver Se concentrations that were higher ($P < 0.05$) than the inadequate levels from control, free-choice selenite and injectable selenite. Whole blood Se was adequate (> 100 μg/L) for all treatment groups at calving, 30 and 90 d postpartum. At 205 d postpartum, cows receiving injectable selenate and both free-choice treatments were adequate in whole blood Se, while controls and cows receiving injectable selenite had inadequate whole blood Se. Cows receiving Se yeast had higher ($P < 0.05$) colostrum Se than other treatments. No differences were observed in milk Se at 30 and 90 d postpartum among treatment groups, however, both free-choice and the injectable selenate treated cows had milk Se numerically higher than controls and cows receiving

injectable selenite. At weaning (205 d postpartum), cows receiving Se yeast had at least two-fold higher ($P < 0.05$) milk Se than cows receiving other treatments.

Cows utilized in this study calved in early January 2003 and effects of Se supplementation on performance and blood and tissue Se concentrations of their calves were also compared and evaluated. No differences in calf weight at birth or weaning were observed among treatment groups. Average daily gain was higher ($P < 0.05$) for calves from dams that received Se via free-choice minerals. Calf whole blood Se was determined at birth, 30, 90 and 205 d of age. Testicles were collected at birth from males and a liver biopsy was performed on all calves at d 205 for liver Se determination. At birth, calf blood Se was in the adequate range (>100 μg Se/L) for all treatments. At d 30 and d 90, control, both injectable products, and free-choice sodium selenite treatments produced calf blood Se in or near the adequate range and Se yeast treated calves remained well above adequate range at 166 and 182 μg Se/L, respectively. At 205 d, whole blood Se concentrations for control and both injectables were in the deficient range (< 50 μg Se/L), calves from the free-choice sodium selenite treatment were marginally deficient (< 75 μg Se/L) and the Se yeast group had blood Se well above adequate at 188 μg Se/L. Selenium in calf testes ranged from 162 to 210 μg Se/kg and did not differ among treatments. Liver Se taken at weaning ranged from 297 to 1321 μg Se/kg and calves from the two free-choice treatments had Se concentrations higher ($P < 0.05$) than both injectable treatments and the controls. Positive correlations ($r = 0.57$ and 0.64; $P < 0.01$) between calf liver and whole blood Se and cow whole blood Se were observed. Correlation between concentration of Se in calf whole blood and in cow milk was also positive ($r = 0.49$; $P < 0.002$).

In conclusion, ewes under our experimental conditions and during the stresses of production were able to tolerate up to 20 mg/kg dietary Se as sodium selenite for 72 wk. These findings suggest the maximum tolerable level of inorganic Se for sheep to be much higher than 2 mg/kg as was suggested previously. Experiments which are longer in duration are necessary to clearly define the maximum tolerable level. Furthermore, our results suggest that ewes consuming up to 20 mg/kg inorganic Se can give birth to normal lambs and that the lambs do not suffer from Se toxicosis before weaning. Selenium as sodium selenite can be fed to ewes at concentrations greater than the current maximum tolerable levels (2 mg/kg) without adversely affecting their offspring. Wether sheep, under our experimental conditions, tolerated up to 40 mg/kg dietary Se as sodium Se and Se yeast for 60 wk, though differences in response to Se source were observed. These results further indicate that the current maximum tolerable level of Se, regardless of source, is much higher than the current estimate of 2 mg/kg and the range between optimal and toxic Se levels is not so narrow. On the contrary, using a maximum tolerable level for Se of 10 mg/kg and 0.1 as the Se requirement, then the range between requirement and toxic level is 100 units. At that range, Cu, Mn, Co, Fe, and Zn then all have a more narrow range from requirement to toxic level than does Se.

In a beef cow-calf herd, Se supplementation with organic or inorganic Se via free-choice minerals or injectable selenate maintained adequate Se concentrations in whole blood, plasma, and liver for 365 d. However, inorganic Se was limited in its ability to increase milk Se, whereas Se yeast increased milk Se at parturition and at weaning. Supplementation of Se as Se yeast to brood cows produced adequate calf blood Se throughout our experiment, whereas control and the other treatments produced calf blood

Se concentrations that were mostly in the marginal or deficient range. Supplementation of Se, as Se yeast, to the cow herd is an effective means of maintaining adequate blood Se in pre-weaned calves and possibly increasing calf ADG.

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BIOGRAPHICAL SKETCH

Paul Armand Davis was born in Winchester, Tennessee on July 11, 1973. The fifth-generation stockman is the elder son of Dr. Donald J. and Carol Davis and has one younger brother, Ryan. He lived in Belvidere, Tennessee, until the age of 3, and then moved to Crossville, Tennessee, where he graduated from Cumberland County High School in 1991. Throughout elementary and high school, Paul was heavily involved in numerous 4-H and FFA livestock projects, was recognized as Cumberland County FFA's Star Greenhand and traveled to Japan as a 4-H exchange student. In January 1992, he began work on a Bachelor of Science in Agriculture at Tennessee Technological University and graduated in May 1996.

As an undergraduate in the animal science concentration, Paul became active in the Block and Bridle Club and held the office of club president. He was honored as Pledge of the Year by his Kappa Sigma Fraternity brothers in 1993, was inducted into the Delta Tau Alpha Agricultural Honor Society, and served on the Animal Science Academic Quadrathlon team in 1994. Paul received the first Tennessee Cattlemen's Association Future Cattle Industry Leader Scholarship, the Joe Scott Memorial Scholarship, and the W. Clyde Hyder Animal Science Award for outstanding senior in animal science.

Following graduation, Paul worked in the livestock industry at People's Stockyards in Cookeville, Tennessee, East Tennessee Livestock Center in Sweetwater,

Tennessee, and with Moorman's Inc. as a sales representative, all while preconditioning and shipping cattle in partnership with brother Ryan.

Paul began work on a Master of Science in ruminant nutrition and beef cattle management in August 1999 at the University of Florida. During his studies, he became involved in Florida Blue Key, Omicron Delta Kappa, Alpha Zeta, Savant UF, Gamma Sigma Delta, Sigma Xi, American Society of Animal Science, and the Animal Sciences Graduate Student Association. He held a teaching assistantship and served as course coordinator for the large animal practicum undergraduate course. In 2001, Paul received a travel fellowship to the International Livestock Congress in Houston, Texas, and was recognized as Graduate Student of the Year in the Animal Science Department. In August 2001, Paul entered a doctoral program in ruminant nutrition concentrating on minerals at the University of Florida and received his Master of Science in August 2002.

As a doctoral student, Paul was the first graduate student invited as a speaker for the Florida Ruminant Nutrition Symposium and gave an invited talk at the AFIA Liquid Feed Symposium in Indianapolis, Indiana. He aided in the teaching of the graduate Vitamins, and Mineral Nutrition and Metabolism courses by giving lectures in the absence of his advisor, Dr. McDowell. Paul was elected to the University of Florida Hall of Fame in April 2003, named the Graduate Student of the Year in the College of Agricultural and Life Sciences in November 2003, and graduated with his Master of Agribusiness in December 2003. During his time at the University of Florida, Paul cultivated a great appreciation for Gator Football, attended 47 games, and never missed a game at Ben Hill Griffin Stadium. Paul was undecided on plans for the future and his possibilities included academia, industry, or production agriculture.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Lee R. McDowell, Chairman
Professor of Animal Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Timothy T. Marshall
Professor of Animal Sciences

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Claus D. Buergelt
Professor of Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 2004

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